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EDITORIAL

TIMES AND CIRCUMSTANCES

A PERUSAL of ancient veterinary literature will reveal a profound conflict of opinion between the men of the past and the present as to their interpretation of the true nature or natural history of disease.

Their different outlook can be explained by the simple assertion that the former sought for an explanation on the realms of conjecture and philosophical dissertation whereas their modern counterpart has at his disposal a great array of facts and the ways and means of adding to that store.

The amount of factural knowledge available to the modern worker is stupendous and it is for ever growing. In the main this is a good thing but it has its defects. When information is on tap and can be made available by the turn of a page, individual initiative and inquisitiveness may be dulled and the searcher can be swamped in the torrent. In the advance of knowledge many paths have been blazed, they are adequately signposted, the ditches and pitfalls have been bridged and many modern young men can achieve renown and without undue mental strain if they just follow the guide book.

Philosophical thought and discussion are now the province of the "Dreamers" and fact and orthodoxy are supreme, but what a poor world it would be if there were no " Dreamers."

Dreamers see visions and in their meanderings of thought they often enunciate ideas and hypotheses which will stand the most critical scientific appraisement.

(*The February issue of The British Veterinary Journal was published on February 26*)

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All things are relative to one another and to time and circumstance.

When we turn back the pages and read the words of our forebears we have every cause to feel humble and it is impudent to judge them by modern standards. If we do then Stephenson's Rocket and Wright's aeroplane may become objects of ridicule although in their time and place they were the outward manifestation of a surge of genius and knowledge which has not been equalled since.

The circulation within the heart should be as well known to a modern veterinary surgeon or medical man as are the simple addition tables to a schoolboy, yet although much was known of the structure of the body, many aeons passed before Harvey made his great discovery. Although the men of the past had not access to a great store of fundamental scientific knowledge and a galaxy of intricate instruments and appliances their powers of observation were acutely attuned and many recorded their observations with a clarity which is rare to-day. They had " eyes that could see " and their accounts of the signs and symptoms of many diseases are of classical standard.

They were devoted to the experimental method in their efforts to ascertain the true nature of disease and especially so in their search for a cure. Their methods may be classed as empirical when judged by modern standards but in their adventures into the unknown they laid down the basis on which the knowledge of the action and uses of many medicinal agents of proved value is founded.

The *British Veterinary Journal* has been in existence for 80 years. Its columns reflect the great change that has occurred in the art and science of veterinary practice. From time to time we will recall the past by publishing short excerpts from old volumes. The first one, which was published in our last issue, seems to indicate that even in those days there were medical men who posed as authorities on veterinary matters.

GENERAL ARTICLES

PRELIMINARY OBSERVATIONS ON A FLOCCULATION TEST IN STUDIES ON BLACKLEG VACCINE

By E. P. LINDLEY, B.Sc., M.R.C.V.S.

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THE importance of blackleg in Nigeria can be assessed by the fact that this Laboratory makes about one and a half million doses of blackleg vaccine every year. Hitherto, this vaccine has been a formalised anaculture but, with the preparation of concentrated vaccines by growing *Cl. chauvœi* in cellophane sacs (Sterne and Thorold, 1951), some difficulty was experienced in standardising the final product. Dried weights, packed cell volumes, opacity methods and total nitrogen estimations have all been used but these have to be correlated with immunity tests in animals.

Blackleg vaccines have been tested in guinea pigs as a routine measure and the use of these animals has been found to be fairly satisfactory; immunity tests in large animals have to be correlated with these. Hall (1930) drew attention to the fact that local sheep, Plateau or Dwarf-Haired sheep, were not suitable for testing blackleg vaccines "as their susceptibility was most unreliable." Sheep purchased locally have, in some instances, withstood a challenge of approximately 50 minimum lethal doses although other sheep have been very susceptible. Sheep, unlike cattle, are not vaccinated against blackleg as a routine measure in Nigeria and hence local sheep do not owe their resistance to vaccination, but probably to exposure to infection; blackleg is widespread and common on the Plateau (Annual Report, 1930, *et seq.*) Cattle are used in a "non-pathogenicity test" on the vaccine but their use in tests involving challenge is vitiated by several factors; their history is unknown, they may have been vaccinated, they are relatively expensive and the disposal of their carcases is difficult.

Sheep have proved suitable as experimental animals in blackleg vaccine tests in many parts of the world (e.g., Hupbauer, 1951). It was desirable that sheep* should be used in the routine tests and therefore it was essential that a serological test should be utilised to select blackleg-susceptible sheep in locally purchased lots.

The agglutination test has been used in antigenic studies of *Cl. chauvœi* by Henderson (1932) and Roberts (1931). The preparation of stable bacterial suspensions presents some difficulty, which is a disadvantage. Thorold (1953) has shown that a slide agglutination test is of limited value in assessing the relative efficiency of blackquarter vaccines.

* All the sheep referred to in this paper were Nigerian Dwarf-Haired sheep. The cattle were white Fulani Zebus.

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Because of these difficulties with the agglutination test and because the precipitin test is capable of refinement to quantitative chemical standards this latter test was adopted for initial experiments. Experiments were made to establish the relationship between flocculating antibodies and the immune state of the animal. It was hoped to verify that cattle and sheep vaccinated with blackleg vaccines gave a positive reaction to the flocculation test and that non-vaccinated cattle and sheep of known history were negative to the test. Studies also included the verification of the specificity of the antigen and that it reacted with *Cl. chauvœi* antibodies only. It was expected that some observations made might show that the varying susceptibility of locally purchased sheep was reflected in their flocculation titres and that the flocculation titres could be correlated with the results of the challenge experiments. The development of flocculating antibodies following vaccination was also observed.

Methods

Bacterial suspensions of *Cl. chauvœi* were prepared by growing a Vom vaccine strain E.65* at 37° C. for 72 hours in a sac made from ¾ in. diameter Visking cellophane casing suspended in meat piece broth. The bacteria were harvested, washed three times in sterile, normal saline and suspended in distilled water..

Initially the suspension so obtained was subjected to supersonic vibrations of a frequency of 1 m. c/s. and a power output of 6 watts for varying lengths of time, but more antigen was not present after one hour's bombardment than in the untreated suspension left for 24 hours in the refrigerator. Similarly, 48 hours' grinding with agate marbles in a ball mill did not increase the concentration of the antigen.

The suspensions were preserved with 0.1 per cent merthiolate and the bacterial bodies separated off by centrifugation at 7,000 revs. per minute for 20 minutes; the clear supernatant fluid was used as the antigen and was standardised against serial dilutions of known positive serum.

Neither the filtrate of the original growth, bacterial washings, digests of the suspension, nor the product isolated by adding 60 per cent ammonium sulphate and dialysing were as satisfactory antigenically as the material prepared as above.

For an accurate determination of the titre of any particular serum it is necessary to ascertain the optimum ratio between antigen and antibody and this is best done by examination of the supernate after flocculation, for excess antigen or excess antibody. However, satisfactory comparative results can be obtained more easily by a visual examination for flocculation and an estimation of the optimum ratio of antigen and antibody.

After some exploratory tests it was found that mixing a 1 :20 dilution of antigen with serial dilutions of serum a comparative value could be obtained by noting the highest dilution of serum at which flocculation occurred. This will

* This strain is probably N.C.T.C. 3764.

occur in the antigen excess zone but it may be assumed that this antigen excess is constant with any one antigen-antibody system. The antigen and serum dilutions were adjusted in an endeavour to make the test sensitive to low titre sera and to eliminate negative sera.

Some sedimentation, which confused the reading of the test, occurred with sheep sera and, very occasionally, with bovine sera, and a serum control in which the final concentration of serum was 1 :2 was necessary for every serum tested. It was necessary to add 0.1 per cent merthiolate to serum controls. A half concentration of normal physiological saline in distilled water was used as a diluent; incubation at 37° C. produced flocculation in more tubes than incubation at 56° C. However, antigen and sera could each be heated to 56° C. for half an hour without interfering with the reaction. Incubation at 56° C. increases the solubility of the precipitate in antigen or antibody when either are in excess (Landsteiner, 1947), and therefore flocculation occurs only in those tubes in which the concentration of antigen and antibody are nearer to the optimum ratio. Incubation at 56° C. was not used as it did not favour the differentiation of low titre and negative sera. In all tests a negative and a positive serum were included as controls.

The antigen appeared to be stable. No double zones of flocculation were apparent.

Two phenomena, common to precipitin reactions, were noted; the amount of floccular deposit varied even with sera of the same titre and the number of tubes in which flocculation occurred was not constant with sera of the same titre.

The technique finally adopted was as follows:—Serial dilutions of sera were made in nine tubes with final dilutions from 1 :2 to 1 :512. The antigen added to each tube made a final dilution of 1 :40. Half concentration of normal physiological saline was used as diluent and 0.1 per cent merthiolate was used to control contamination. A serum control with a final dilution of 1 :2 was included and the tubes were incubated in a water bath at 37° C.

Three degrees of reaction were observed; a flocculation of bluish-grey cotton-wool like material in suspension; a turbidity in which particles were present but not recognisable as floccules; an opacity in which the mixture appeared homogeneous.

Sedimentation occurred progressively when the tubes were left to stand. Three readings were therefore made, at 6 hours, 9 hours and 18 hours of which the middle reading was the most valuable. The titre in these records represents the highest dilution of serum at which flocculation was detectable.

Experiment 1

The difference in the reaction to the flocculation test of vaccinated and non-vaccinated animals.

A. Cattle.

Thirty-six Zebu cattle of known history, bred and reared at the Livestock Investigation Centre, Vom, were tested. Twelve had not been vaccinated and 24 were vaccinated.

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Group 1(a).

Vaccinated subcutaneously with 5 c.c. of blackleg anaculture vaccine on December 9, 1953; repeated on January 30, 1954. Tested on June 26, 1954.

Number	Titre	Number	Titre	Number	Titre
313	1:16	317	1: 2	325	1.32
314	1: 8	319	1:64	327	1: 2
315	1:16	322	1:16	328	—
311	1: 8	323	1: 4	329	1: 2
				324	1:16

Group 1(b)

Vaccinated subcutaneously with 5 c.c. of blackleg anaculture vaccine on December 9, 1953; repeated on January 30, 1954. Tested on August 7, 1954.

Number	Titre	Number	Titre
339	1:16	347	1:32
340	1:16	348	1:32
341	1: 4		

Group 1(c)

Vaccinated subcutaneously with 5 c.c. of blackleg anaculture vaccine on March 13, 1954. Tested on August 7, 1954.

Number	Titre	Number	Titre
342	1:16	345	1:32
343	1: 8	346	1:16
344	1: 4	349	1: 8

Group 2.

Non-vaccinated cattle. Tested on June 26, 1954. Numbers 308, 309, 310, 312, 316, 318, 320, 321, 326, 330, 331, 332, were all negative.

B. Sheep.

Thirteen sheep were tested on June 21, 1954; three of these had been vaccinated 21 days previously with 10 c.c. of blackleg anaculture vaccine subcutaneously. Ten had not been vaccinated, and their history was known, having been bred and reared at the Livestock Investigation Centre, Vom. The results were as follows:—

Group 1.

Vaccinated sheep.

Number	Titre
48	1:256
53	1:256
71	1:256

Group 2.

Non-vaccinated sheep.

Numbers 102 to 111 inclusive were negative.

Experiment 2

The Specificity of the Test.

It was necessary to verify that the reaction was specific for the antigen-antibody mixture of *Cl. chauvœi* and that there was no cross reaction with related Clostridial antibodies. Hyperimmune sera against *Cl. septicum, Cl. œdematiens* "A" and "B," *Cl. welchii* "B," "C" and "E" were tested and none gave a positive flocculation with the *Cl. chauvœi* antigen.

Experiment 3

The flocculation titres of sera from locally purchased sheep.

As groups of locally purchased sheep vary in their susceptibility to challenge with blackleg organisms, flocculation tests were carried out to see if this variation was reflected in their flocculation titres.

Group 1.

Fifteen sheep obtained from Mungo Arna were tested on June 17, 1954.

Number	Titre	Number	Titre	Number	Titre
21	1:16	63	1: 4	86	1: 2
31	1:16	64	1: 4	91	1: 4
40	—	72	1:16	92	—
53	1: 4	80	—	93	1: 2
98	1: 4	85	1: 8	94	1: 2

Group 2.

Fifty sheep obtained from Maidontoro were tested on June 14, 1954. Six gave some reaction and are given below:—

Number	Titre	Number	Titre
123	1:8	153	1:8
129	1:8	144	1:8
162	1:2	137	1:2

The remainder were negative.

Of 65 sheep which were purchased locally and had not been vaccinated, 18 gave positive flocculation and this is interesting because of the difficulties in using such sheep for the testing of blackleg vaccine

Experiment 4

The inoculation of sheep of known flocculation titres with cultures of Cl. chauvœi.

(*a*) Six sheep from those tested above were selected; two had given titres of 1:16, two of 1:256 and two were negative. These sheep were challenged on June 22, 1954, with a mixture of 24 hours cultures of three virulent Nigerian strains of *Cl. chauvœi,* Numbers 1, 7 and 54. Three of the sheep (one of each

pair) were given 2 c.c. intramuscularly and three were given 5 c.c. intramuscularly; 0.1 c.c. given intramuscularly was known to be fatal to susceptible sheep. The results were as follows:—

Number	Titre	Challenge dose	Result
21	1:16	2 c.c.	S
31	1:16	5 c.c.	D_2
48	1:256	2 c.c.	S
71	1:256	5 c.c.	S
40	—	2 c.c.	D_1
80	—	5 c.c.	D_1

D_1 = died within 1 day.

S = survived.

Sheep 21 and 31 probably owed their resistance and flocculation titres to natural exposure; it was not due to vaccination. Sheep 48 and 71 owed their flocculation titres to vaccination having been negative before this first investigation.

(*b*) Eight sheep, negative to the flocculation test, were vaccinated and, together with two controls, were retested after three weeks. They were then challenged with a mixture of 24 hours cultures of virulent *Cl. chauvœi*, as detailed in (*a*) above. The titres of these sheep before challenge and the results of the subsequent inoculation were as follows:—

Number	Titre	Challenge dose	Result
102	1:64		
103	1:32	2 c.c.	D_5
109	1:128	2 c.c.	S
110	1:8	2 c.c.	D_2
140	—	2 c.c.	D_1
104	1:32	5 c.c.	S
105	1:32	5 c.c.	D_2
108	1:32	5 c.c.	S
111	1:64	5 c.c.	D_5
132	1:2	5 c.c.	D_2

D_2 = died in two days.

S = survived.

These sheep were in relatively poor condition at the time of challenge. It has been noticed in Vom that, when animals in good condition are challenged and are still alive on the fifth day, they usually survive. Factors other than blackleg are frequently involved in animals dying after four days.

(*c*) Fifteen sheep previously tested and found negative were vaccinated with blackleg vaccine which had been prepared by growing the organisms in a cellophane sac. The sheep were vaccinated as recorded below, the dose being increased tenfold for each lot.

The titres of the sheep at 21 days are shown. The sheep were challenged at 23 days with a mixture of cultures of local strains of virulent *Cl. chauvœi*, as described previously. The dosages of the challenge and the results are noted below.

Number	Vaccinated	Titre	Challenge dose	Result
113	0.01 c.c.	1 :16	5 c.c.	D_1
114	of blackleg	1 :8	5 c.c.	D_1
115	vaccine	—	2 c.c.	D_2
116	0.1 c.c.	1 :8	5 c.c.	D_1
117	of blackleg	1 :32	5 c.c.	S
118	vaccine	1 :4	not challenged	—
119	1 c.c.	1 :512	5 c.c.	S
120	of blackleg	1 :128	5 c.c.	S
121	vaccine	1 :32	5 c.c.	S
122	10 c.c.	1 :128	5 c.c.	S
124	of blackleg	1 :64	5 c.c.	D_3
125	vaccine	1 :64	5 c.c.	S
127	100 c.c.	1 :4098	10 c.c.	S
128	of blackleg	1 :8196	10 c.c.	S
131	vaccine concentrated centrifugally	1 :512	5 c.c.	S
146		—	0.1 c.c.	D_2
154	not	—	0.2 c.c.	D_2
161	vaccinated	—	2 c.c.	D_2

D_2 = died after two days.

S = survived.

The results of this experiment are relevant to the standardising of vaccines, as they give a broad indication of the relationship between the amount of flocculating antibody formed and the quantity of antigen inoculated.

(*d*) A further vaccine test is recorded in which the flocculating titres of the sheep showed up two inferior batches of experimental concentrated vaccine and this was confirmed by challenge. Again only negative sheep were vaccinated and these, together with the controls, were retested at 21 days and challenged

at 23 days with a mixture of 3 cultures of virulent *Cl. chauvœi* as in (*a*) above.

Number	Vaccinated	Titre	Challenge dose	Result
136	5 c.c. of	1 :128	All were	S
141	blackleg	1 :256	given 2 c.c.	S
143	vaccine B/12/54	1 :128	„	S
135	5 c.c. of	1 :64	„	S
138	blackleg	1 :64	„	D3
151	vaccine B/14/54	1 :256	„	S
133	1 c.c. of	1 :8	„	D2
139	1/20 dilution			
145	of blackleg	1 :16	„	D2
	CBQV/19/54	1 :32	„	S
147	1 c.c. of	1 :16	„	D2
149	1/20 dilution	1 :32	„	D2
152	of blackleg	1 :64	„	S
	CBQV/20/54			
157	not vaccinated	—	„	D1

D2 = died after two days.
S = survived.

Experiment 5

The flocculation test and the development of antibodies after vaccination with blackleg (formalised anaculture) vaccine.

A. Cattle.

Twelve cattle which had not been vaccinated and had been bred and reared at the Livestock Investigation Centre, Vom, were all negative to the flocculation test. (cf. Experiment 1, Group 2.) These cattle were vaccinated with 10 c.c. of blackleg formalised anaculture vaccine on July 24, 1954, and were bled at various intervals after vaccination. Their flocculation titres were:—

Number	Flocculation Titres on days following vaccination									
	1	2	3	4	5	6	7	8	14	21
308	—				1 :64				1 :64	1 :256
309	—				1 :128				1 :256	1 :128
310	—				1 :512				1 :256	1 :256
312		1 :2				1 :32			1 :64	1 :512
316		—				1 :128			1 :512	1 :512
318		1 :2				1 :64			1 :32	1 :16
320			1 :2				1 :64		1 :32	1 :32
326			—				1 :128		1 :512	1 :512
330				1 :16				1 :512	1 :512	1 :512
331				1 :16				1 :512	1 :128	1 :512

B. Sheep.

Eighteen sheep, with negative reactions, were vaccinated on August 9, 1954, with 10 c.c. of blackleg formalised anaculture vaccine. Flocculation test results on their sera on the days following vaccination were:—

Number	Flocculation Titres on days following vaccination								
	1	2	3	4	5	6	7	8	9
106	—			1:64			1:256		
107	—			1:32			1:128		
162	—			1:64			1:128		
164	—			1:64			1:512		
165	—			1:64			1:512		
166	—			1:64			1:512		
150		1:4	1:4		1:128			1:512	
155		1:2	1:4		1:128			1:128	
168		1:4	1:4		1:512			1:512	
169		—	—		1:128			Not tested	
172		—	—		1:128			1:128	
173		—	1:8		1:128			1:512	
159						1:32			1:512
175						1:256			1:512
176						1:128			1:64
178						1:512			1:512
179						1:16			1:128
181						1:64			1:32

The rate of increase of these circulating antibodies was in most cases greatest between 72 hours and 120 hours after vaccination.

Discussion

All sheep which were negative to the flocculation test and have been challenged were susceptible to blackleg. This is very important at this Laboratory because sheep can now be used in the routine testing of blackleg vaccine.

From the foregoing experiments it is also apparent that the flocculation test gives some indication of the level of protective antibodies in vaccinated sheep. Similar experiments on cattle were not made and this relationship between flocculating antibodies and protective antibodies has not been confirmed in them. If this relationship is substantiated, then a test is available which will enable a thorough investigation of immunity in relation to blackleg to be made.

The flocculation test, as carried out in these experiments was simple; it is a practical test and although it might be desirable that absolute rather than comparative methods should be adopted, it is obvious that such standards would have made the test too cumbersome for routine work.

The nature of the antigen was not investigated and it is for this reason that the test is described as a flocculation test rather than as a precipitin test; Mason (1936) was unable to obtain flocculation with toxin and antitoxin mixtures. He

was unable to find any correlation between the amount of circulating antitoxin in an animal and the power of the animal to resist the inoculation of living cultures, whereas the reacting antibody in these experiments was related to protective antibodies. This suggests that it is not antitoxin, although the reaction appears to be of the Ramon type (toxin-antitoxins) (Kabat and Mayer, 1948).

Guinea pigs were used in some of the initial tests and their sera reacted in a similar manner to those of sheep and cattle. Guinea pigs which had not been vaccinated were negative and vaccination produced positive titres and after a challenge the titres soared. The main drawback in working with guinea pigs was the difficulty of obtaining sufficient blood for the tests without killing them

Since its introduction in this Laboratory the flocculation test has been used, to assess the potency of batches of vaccines, and as a diagnostic test. Although it will not replace challenge experiments it may reduce the number which are necessary. Its value as a diagnostic test appears to lie in the ready differentiation of infections of *Cl. chauvœi* when *Cl. septicum* or *Cl. œdematiens* are also involved.

Summary

A flocculation test is described which has been used successfully for the selection of blackleg-susceptible sheep. From the limited number of experiments performed it appears that the test gives an indication of the level of the protective antibodies in sheep and will therefore be useful in studies of immunity in blackleg. Results which show the development of the flocculating antibodies following vaccination are given. The use of the flocculation test as a diagnostic procedure is noted.

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THE CULTURE OF EARTHWORMS AND THEIR INFECTION WITH *METASTRONGYLUS* SPECIES

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THERE is little published work concerning the culture of earthworms for any specific purpose, though numerous workers have perfected methods. The present study forms part of an investigation on the lungworms of pigs. It was necessary to devise a method for culturing different species of earthworms in order that a constant supply of worms free from lungworm larvæ could be ensured. The method employed is a modification of one suggested by Mrs. E. Matthews, County Pests Officer, Anglesey, who has cultured *Lumbricus terrestris* Linn. for mole destruction, using wooden boxes and a medium similar to that used in the present study. Darwin (1882) reared worms in soil for short periods for purposes of observation, and Nicholas (1949) cultured worms to provide food for *Ornithorhyncus anatinus* (duck-billed platypus). In none of these records is reference made to individual species of worms used.

For this investigation it was necessary to identify individual earthworm species and to be able to culture these as pure cultures, since one aim of the work was to show which species of worms could be infected most readily with *Metastrongylus*. A brief description of the method has been published elsewhere (Dunn, 1954, Dunn and White, 1954).

Material and Methods

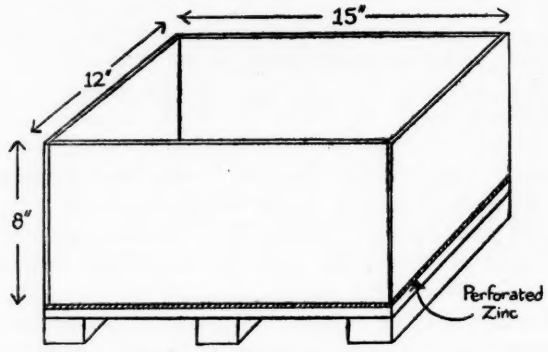
Earthworms were reared in strong wooden boxes 15 inches long, 12 inches wide and 8 inches high, with a base of perforated zinc (perforations 2 mm. diameter), supported by wooden struts and surrounded by a wooden frame. (Fig. 1.)

Equal parts of fæces (cow and goat), good loam and leaf mould were well mixed and sieved through an ordinary garden sieve of $\frac{1}{4}$ in. mesh. Any earthworms seen were removed. This medium was then spread on trays to a depth of about 1 in. and steam-sterilised for 3 hours at 100° C. in order to kill any invertebrates left in the soil. A layer of steam-sterilised hay about 1 in. thick was spread on the bottom of each container, which was then filled to within 1 in. of the top with sterile medium, and 225 grammes of maize meal were mixed with each boxful. The boxes with contents were then left to settle for 2 to 3 days. The contents were thoroughly soaked with water immediately before the addition of worms.

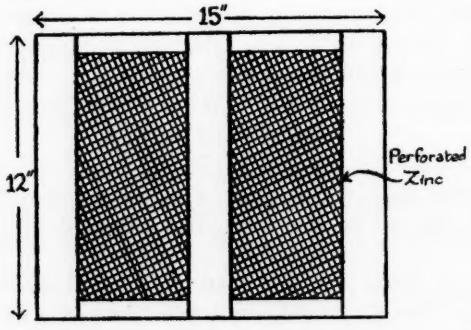
Initially, cultures were started in August, 1952, with a single species, *Eisenia fœtida* Savigny (Syn. *Helodrilus fœtidus*). Fifty mature *E. fœtida* were placed in each of two containers and 50 cocoons of *E. fœtida* were placed in each of two others. Worms and cocoons were obtained from a worm farm in Cornwall.

Subsequent cultures were started in the same way with adult specimens of

Allolobophora chlorotica Savigny, *A. caliginosa* Savigny (Syn. *A. turgida*), *A. terrestris* Linn., *L. rubellus* Hoffmeiseter, and *L. terrestris* Linn., all of which are common inhabitants of pig pastures in the Wirral area of Cheshire. The specimens were collected from small plots of land to which no pigs had access, and a proportion of specimens collected were examined to ensure freedom from infection by lungworm larvæ.



(a.) Side view of Box.



(b.) Base of Box, showing arrangement of Frame and Struts.

Fig. 1

The cultures were kept initially at a temperature of 16° C. but were later removed to a stall where the temperature rarely exceeded 10° C. The higher temperature ensured faster and continuous breeding; at the lower temperature, once established, the cultures replaced themselves satisfactorily with the exception of *L. terrestris* which virtually died out. Possibly this was due to there being insufficient specimens in the culture to ensure continued breeding, since only 30 specimens were found. This earthworm is not the "common" earthworm by which name it is so frequently known. The two commonest species are probably

A. terrestris and *E. fœtida,* the former in pasture, the latter in manure heaps and other habitats with a high content of decaying organic matter.

At first the cultures were fed weekly with a mixture of 1 lb. treacle to 1½ gallons water. It was later found necessary to feed only once a fortnight as long as the cultures were sufficiently moist. Each box received 1 pint of the solution, applied with a watering-can.

Once a month each box received ½ to ¾ lb. minced fodder beet and tea-leaves which, together with the treacle, replaced organic matter and carbohydrate in the medium and were utilised as food by the earthworms.

Methods of infection of earthworms with lungworm larvæ.

The lungworms of pigs, two of which, *Metastrongylus apri* Gmelin and *M. pudendotectus* Vostoskov, occur in Britain, have an indirect life cycle. Embryonated eggs are passed out of the pig in the fæces and must be ingested by an earthworm before further development can occur. Within the anterior part of the gut of the earthworm the eggs hatch and the emerging first stage larvæ migrate into the wall of the alimentary canal and sometimes into the dorsal blood vessel. Two moults take place and the resulting third stage larvæ are infective to pigs eating the infected earthworms whilst rooting in the ground. Development is completed within the pig. (Hobmaier and Hobmaier, 1929, Schwartz and Alicata, 1934.)

The earthworm cultures in this study were used

(*a*) To find which, if any, could be most readily and abundantly infected with *Metastrongylus* species.

(*b*) To obtain a supply of infected earthworms in order to establish *Metastrongylus* species in laboratory animals for possible diagnostic and therapeutic work.

Lungworm eggs for infection or earthworms were obtained from two sources:—

(*a*) *Infected pig fæces.* This did not give a very high concentration and was used only for purposes of testing the viability of eggs from infected pigs.

(*b*) *Adult female lungworms.* These were removed from freshly-killed pig lungs at bacon factories, washed in normal saline and chopped finely in tap water. The resulting concentrated suspension of eggs was found satisfactory for infection of earthworms. This is a similar method to that used by Hobmaier and Hobmaier (1929) and Schwartz and Alicata (1934). Plant pots 10 in. in diameter were filled to within 4 in. of the top with culture medium. Earthworms to be infected were placed in the pots, and the suspension of *Metastrongylus* eggs in tap water was poured on top of the soil.

When pig fæces was used, this was mixed with an equal volume of sterile soil, and earthworms to be infected were placed in plant pots in this mixture. Infections obtained in this way were never as heavy as those obtained by adding eggs in tap water to the soil.

A third method of infecting earthworms was by introducing known volumes of suspensions of *Metastrongylus* eggs through the mouth into the œsophagus with

capillary pipettes. This method was used for testing the viability of small numbers of eggs, as these would probably have been "lost" if put directly into soil and left to chance ingestion by an earthworm.

Method of examining earthworms for lungworm larvæ.

Live infected worms were opened along the dorsal surface from the mid-body to the head. The anterior parts of the gut (œsophagus, crop, gizzard and first part of intestine) were removed, opened and washed free from contents. The tissues were placed between two glass slides, squashed and examined under a low power stereoscopic microscope. Blood vessels and reproductive organs were similarly examined. Larvæ, when present, were readily visible. They were found always in the crop and posterior part of the œsophagus, occasionally in the dorsal blood vessel in large numbers, but never in the reproductive organs, gizzard or intestine. For purposes of infecting experimental animals, therefore, either the crop, œsophagus and blood vessels of dissected earthworms were used, or else that part of the body containing these organs.

The calciferous glands associated with the œsophagus were not always sufficiently well developed to be recognised but when they were found in infected earthworms they always contained larvæ of *Metastrongylus* spp.

Discussion

Eisenia fœtida was the most successful and easy earthworm species for culture and for infection with *Metastrongylus* spp. It thrived well at low and at high temperatures, bred rapidly, and though not found on pig pasture, was much the easiest species to infect heavily with *Metastrongylus* spp.

Allolobophora terrestris and *A. caliginosa* were not difficult to culture and to infect. They were the commonest inhabitants of pig pastures. The remaining species were more difficult to culture. Only *L. terrestris* has not yet been successfully infected with *Metastrongylus* spp., and this may well be due to the shortage of specimens available for testing.

The culture method adopted in the present study appears to present optimum conditions for the development of *Eisenia fœtida* whose natural habitat is manure and compost where the amount of decaying organic matter is high. If a study were made of the individual requirements of each of the other species, more suitable culture media for them might well be found. Their natural habitat is different from that of *E. fœtida,* for they live in pasture where the decaying organic matter content is lower.

Schwartz and Alicata (1934), in their work on *Metastrongylus,* found that *E. fœtida* was the most readily infected species of earthworm. They also state that this species was common on pig pastures. The author has not found it on pig pasture but artificial infections with *Metastrongylus* spp. have been invariably successful. The names *Lumbricus terrestris major* and *minor* used by Hobmaier and Hobmaier (1929) have no zoological standing, so that no comparison with their results is possible.

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All species bred faster at 16° C. than at 10° C. or less. At the higher temperature they also bred throughout the winter months whereas breeding ceased in winter at low temperatures.

First stage larvæ were generally found in *E. fætida* 3 to 4 days after the worms were put in infected soil, second stage larvæ after 9 to 10 days and third stage larvæ after 11 to 12 days. These results are similar to those of Schwartz and Alicata (1934) who also give descriptions of the various stages in the life history of each of the three species of these nematodes.

The earthworms in the present study were left in the infected medium for at least four weeks in order to ensure a larger proportion of infective larvæ being available for experiments.

Summary

(1) A method of culture for earthworms is described with details of containers, medium, food and species of earthworms used.

(2) Methods of infecting earthworms with *Metastrongylus* spp. are described, together with the method of examining infected earthworms for lungworm larvæ.

(3) Of six common species of earthworms, *Eisenia fætida* Savigny was the easiest to culture and infect. Time relationships in the development of lungworm larvæ in this species are given.

Acknowledgments

I should like to thank Professor E. G. White for valuable help and criticism throughout this work, Mr. R. B. Griffiths for reading the manuscript, Mr. H. P. Harding and Mrs. E. Matthews for help in my preliminary investigations on earthworm culture, and Miss M. A. Gentles for able and willing technical assistance.

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FURTHER OBSERVATIONS ON CEREBROSPINAL NEMATODIASIS IN ANIMALS

II.—The Problems of Reinfection by Nematodes and Clinically-Silent Cases

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Shoho (1951, 1952 and 1954) and Innes, *et al.* (1951, 1952, 1953 and 1954) have recorded most aspects of cerebrospinal nematodiasis. Ishii, *et al.* (1953) reported results of a transmission experiment (in goats), a repetition of studies made by the Special Research Committee in Korea (on sheep, 1939-1943)** and by Itagaki, *et al.*, in Japan on foals (1948).† From the work now printed in English no doubt should remain regarding the common occurrence and nature of a disease, caused by the wandering of immature nematodes in the nervous system, which may terminate in an acute or subacute clinical paralysis. Other issues were raised by Innes and Shoho. The most important was that this disease might have occurred in animals (and man) in areas remote from the Orient, and had never been identified. The malacic focus may be difficult to locate and identify, and the causal worm may not be found easily except in acute cases, and then not necessarily in the neural lesion. With these possibilities in mind, pathological examinations have revealed the existence of a disease in goats in India (Innes, 1953) and horses in Ceylon (Innes and Pillai, 1955) which almost certainly is the same. Conversely, a paralytic disease in sheep in Kenya, reported by Plowright (1954) and Innes and Plowright (1955) is not nematodiasis but a peculiar focal symmetrical spinal poliomalacia of unknown etiology, although there might be some superficial clinical similarity.

It is clear that this helminthic neurologic disease could be confused clinically with other disorders, particularly with the virus encephalitides, in those countries where they are enzootic. There are other problems: (*a*) Can animals be infected once, recover and be re-infected later in the same season, or the following year? (*b*) Is it possible for an animal to be infected, but for the worms to be located in a neuroanatomical region in which damage would be so slight as not to produce clinically apparent disease, or only minor, almost indiscernible, signs? If either can be answered in the affirmative, there are

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** Reported at the International Veterinary Congress, Stockholm, August, 1952, by Professor Y. Ochi, at one time the chief of the Research Committee in Korea.

† The work by Itagaki, *et al.*, is contained in the publication by the Special Research Committee for Lumbar Paralysis of Foals, 1948.

more repercussions, and this paper outlines observations in these directions. There is a relevant publication which concerns the association of an ascaris larva in the brain of a child with lesions of acute anterior poliomyelitis (see Beautyman and Woolf, 1951). They found no naked eye changes in the brain, but thirty sections through the thalamus accidentally revealed a granuloma (0.4 mm. x 0.36 mm.) in the *nucleus ventralis lateralis;* there were periarteriolar hæmorrhages remote from the lesion, but in addition there were changes typical of anterior poliomyelitis in the spinal cord. The unique features relate to the fact that, clinically, the case was one of poliomyelitis, and that any signs which might have been due to a larval granuloma were masked by the viral infection or completely silent. The larva was assumed to be that of *Ascaris lumbricoides,* but the possibility of its being a larva of *Toxocara canis* was not excluded. [The cerebral wandering of larvæ of *Toxocara canis* is well known since the works by Fülleborn (1921) and Yokogawa (1923), and recently Tiner (1953) and Sprent (1953) have confirmed this in mice. The infestation by larvæ of *Toxocara canis* in the liver of children has also been stressed by Beaver, *et al.* (1952), Smith, *et al.* (1953) and Brill, *et al.* (1953)]. In all discussions on the migration of larvæ into the CNS, emphasis is usually laid on intravascular paths, and little consideration given to a direct wandering through tissue into the cranio-vertebral cavity, as in cerebrospinal nematodiasis of animals. Innes, Shoho and Pillai (1952) brought notice to the work of von Brand and Cullinan (1943) who showed that such direct tissue migratory paths were taken by some larvæ in experimental abnormal hosts, and that the nervous system, under these conditions, might then be a strange tissue of attraction for worms. Probably, a cardinal concept of Beautyman and Woolf's study was that the ascaris might have facilitated the poliomyelitis by inducing an immune reaction, or some non-specific effect as described by Findlay and Howard (1950). Innes and Shoho (1952) indicated that a similar relationship between *Setaria digitata* (a cause of neural nematodiasis) and Jap B virus encephalitis in man and animals in Japan could not be ignored, for both are arthropod-borne diseases of the CNS, and occur annually in the same season. The same situation may also apply in Malaya, for recently Jap B virus encephalitis in man and horses has been reported there by British and American workers [Paterson, *et al.* (1952) and Pond, *et al.* (1953)], and *Setaria digitata* has been known to occur since the work of Purvis (1931). In the U.S.A. and some northern countries of South America, where the occurrence of the arthropod-borne viral encephalitis (Hammond and Reeves, 1945) is well known, *Setaria digitata* is being found more frequently in the peritoneal cavity of domestic cattle [see Kaupp (1918), Cameron (1930), Baylis (1936), Gonzales (1946) and Frickers (1948), and personal communications from Dikmans (1951) and Rocha (1953)]. Recent work by Mochizuki, *et al.* (1954) in Japan, proving that a form of experimental cerebrospinal nematodiasis (by larvæ of *Toxocara canis*) could be a provoking factor in initiating Japanese B encephalitis of experimental mice, now support our own suggestions which were based on observations of the natural diseases. According to Mochizuki, *et al.,* the precipitation of the onset of a viral

encephalitis in mice might be explained by mechanical damage to the blood-brain barrier by a worm, enabling virus to achieve direct contact with nervous parenchyma in the stage of viremia, as speculated by Innes and Shoho (1952).

Reinfection by Setaria Digitata

Neural nematodiasis is not a rare bizarre entity either in animals in Korea and Japan, or in goats (and now horses) in Ceylon, or goats (and almost certainly horses as well) in India. The disease has been confused for years with virus encephalitis in Japan. In Ceylon, this complexity did not arise for, apart from rabies, none of the animal virus encephalitides have been identified. In Japan it is generally assumed that the average seasonal incidence of the cerebrospinal nematodiasis may be as high as six to seven per cent of all sheep and goats, and that about ten per cent of all affected cases die; the rest may recover with, or without, residual nervous signs. This figure approximates that obtained in the Fukushima prefecture in 1943, and again in 1951 by one of us (C.S.). A much higher figure was found in one village in the more southern prefecture in Japan, where newly imported* goats are kept; for example, nearly thirty per cent of animals were affected in one village in Gumma prefecture, Hosoya, *et al.*, (1952).

In Japan it is a well-known field observation that many animals are affected seasonally with a minor degree of neurologic disorder manifested by signs of motor weakness with slight impairment of gait or actual inco-ordination, or unilateral drooping ear or eyelid, and that these animals can be kept alive with special care (by suspending them over a straw mat). These signs may not progress, and may gradually become indiscernible.

The animals reported here† were killed for the purpose of our study at Fukushima in 1943. The brains and the cords were fixed in formalin and sections from selected areas were stained with hæmatoxylin and eosin.

Case I (No. 5): Well-nourished ewe, two years old. Nervous signs: nystagmus and ataxia but no fever on the morning, September 7, on which day autopsy was performed. Macroscopic changes consisted of milky white lesions in the spinal cord, distributed bilaterally along the dorsal median septum between C_{1-7}. Microscopically, an area of primary microcavitation surrounded by sclerotic tissue was found in C. The cranial and caudal portion

* This interesting phenomenon of "newly-imported" animals being "more sensitive" to the disease crops up time and again in the history of cerebrospinal nematodiasis—viz., Swiss goats into Japan, Indian goats into Ceylon, Corriedale sheep into Korea, and foreign horses into India over 150 years ago. (J. R. M. Innes.)

† The four cases reported in this paper are selected from a total of forty-six cases, dissected by one of us (C.S.) in 1943. The study on the pathology of all cases with detailed topography of the lesions by S. Yamagiwa, C. Shoho, K. Iwamura, M. Ninomiya and Y. Yamate was in course of publication during the last war, but was interrupted and never completed.

Almost all stages of typical lesions of this disease have been illustrated in the various papers quoted in the introduction, and it is unnecessary to present further photographs. An attempt has, however, been made to depict the various lesions, in these four cases chosen for the purpose of this paper, in a schematic form but giving impressions both of location and type of lesion.

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to this showed secondary degeneration of nonsclerotic stage. Besides these findings, some sections from C_1, C_4 and C_5 showed eosinophilic infiltration around the vessels at the entry of the dorsal median sulcus and lymphocytic and eosinophilic infiltration around vessels and nerve roots extra-durally. At C_7 extra-dural infiltrations contained giant cells.

Comment. Even though no clinical history of previous infection was given by the owner, this case was concluded to be one of re-infection. The first infection had apparently caused the lesion in the dorsal column, for this was certainly more than a day or two old, while the second one would be represented by the cell infiltrations around the vessels in and outside the dura. The ataxic condition noted before the death might have been caused by the wandering nematode in the subdural space, which had escaped identification at dissection. The lesion in the dorsal column at C_4 with the formation of fibres of mesodermal and glial origin was certainly more than a few days old and suggests to us the presence of previous infection, either with no signs, or signs that had been overlooked by the owner.

Case II (No. 11): Well-nourished ram, four years old. Nervous signs were first seen 32 days before killing, when slight slanting of the head, weak hindquarters and indifference to ewes in heat were observed. There was no fever and respiration was normal. Macroscopically, lesions were found in the brain and the spinal cord: (1) a reddish-yellow malacic lesion, extending from the *capsula externa* to the white matter of the *right lobus piriformis;* (2) a hæmorrhagic lesion, irregularly distributed from the *capsula externa* to the *tractus opticus* via *capsula interna* in the left side; and (3) milky white spots in C_1 and $_2$. In lesion (1) fibre formation witth some vessel proliferation was distinct and with lymphocytic infiltration around vessels, while, in lesion (2) the microcavitations contained blood and tissue detritus, and very marked eosinophilic infiltration around vessels (in *capsula externa*); while in (3) at C_1 and $_2$, an old sclerotic lesion was found in the left lateral column and there was a fresh lesion along the ventral median fissure.

Comment. According to the protocol, the case was relatively long standing. The hæmorrhagic lesions, with the finding of perivascular eosinophilic infiltrations, reflect somewhat the wandering of the worm shortly before dissection. The glial and mesenchymal fibre formation in the right lobus piriformis and in the left lateral column of C_1 and $_2$ are grounds for assumption that a previous infection had occurred more than a month before death. It was, therefore, highly probable that the worm which caused these lesions was different from the one which caused the hæmorrhagic malacic lesion in the *capsula externa.* It is noteworthy that the animal could stand and walk with slight nervous signs, even though, pathologically, such severe lesions were found.

Case III (No. 27: Ram, four years old. This was a unique case with a history of nervous signs in the previous year which had been well tolerated by the animal. The nervous signs of the second infection on our observations were inco-ordination and tendency to lie down on the day of killing. Body tempera-

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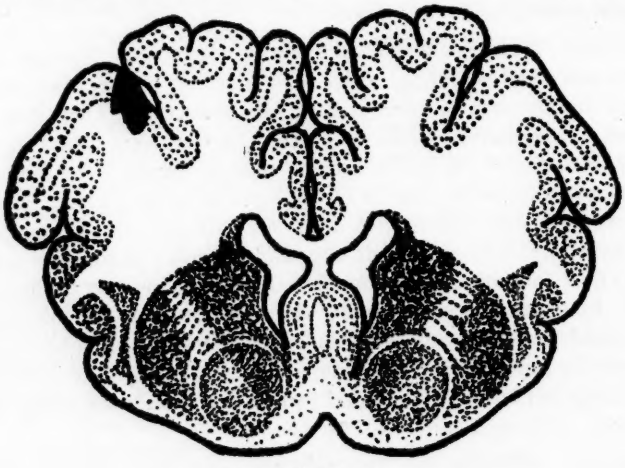
ture was 39.9° C. and respiration was normal. Macroscopically, there was a decrease in volume (atrophy) of the left ventral white column along the whole length of the spinal cord. Microscopically, typical sclerosis with primary micro-activations was found in $C_{1, 2}$, and $_3$. The more caudal portion showed atrophic lesions with resultant deformity. In the right ventral column slight fresh lesions were also found along the ventral median fissure, microcavitation, but without any remarkable glial changes.

Comment. The sclerotic lesions with atrophy of the left ventral white column is clear evidence of old-standing, pre-existent infection one year before. In this case there is doubt that the older lesion alone might have been responsible to provoke the persistent slight nervous signs, but the lesions of the ventral column of the opposite side is also convincing proof of the occurrence of a relatively recent malacia.

Case IV (No. 31): Well-nourished ewe, eight years old. The animal was killed 20 days after the first nervous signs. The sheep was still lying on its side,



Case IV, No. 31
The cerebral lesion at the Globus pallidus level. (View of the nasal cut surface.)

but could keep its head upright at times. There was no fever and respiration was normal. Macroscopically, there were (1) a reddish-yellow malacic lesion in the left cerebral cortex (involving *Sulcus suprasylvius*); (2) milky white streaks distributed irregularly in the dorsal white column of C_1 and $_2$, and in the ventral white column of C_4-T_3; and (3) slight lesions in the right dorsal column of L_3. Microscopically, the lesion in the cerebral cortex showed the *Körnchenzellen* (scavenger cells) stage, in which the damaged tissue was enmeshed by fascicular fibrous masses, and the spaces between them occupied by *Körnchenzellen*. The cavity partly occcupied the white matter. In the lesion of the dorsal column of C_1 and $_2$, fresh cavitations were found with minimal reaction in the surrounding

tissue, while in the left ventral column sclerotic plaques with primary cavitation were found in C_8. The lesion of L_3 was relatively fresh secondary degeneration.

Comment. From the stage of the lesion in the cerebral cortex and in the dorsal column of C_1 and $_2$, these changes were regarded as responsible for the nervous signs before killing. The nature of the lesion in the ventral column of C_8 reflects the existence of an old infection.

General Comment. Even though in all these cases no worms were found in the lesions, nor in the cranio-vertebral cavity, the typical nature of the pathology, the topography of the whole lesions with microcavitations, the tracking relationship to the outside of the nervous parenchyma, the tissue reaction around cavities such as terminal swelling of the axons, cellular increase of mesodermal and glial elements as observed in the colliquative type of necrosis, and the perivascular eosinophilic infiltration, are all convincing enough that these cases were cerebrospinal nematodiasis. The other nervous diseases of domestic animals in the same season of the year are Jap B encephalitis and purulent encephalitis (due to hæmolytic streptococcus which has been reported only in horses). Changes characteristic of either were not found in these sheep cases, nor in all other 42 cases examined under similar circumstances at the same time (1943). Thus, we do not hesitate to conclude that the cases are of nematodal origin, especially by consideration of their peculiar pathological changes. In estimation of duration of lesion we believe that fibre formation of glial and mesodermal origin can be regarded as a reaction of a much later stage than a month or more. Such a reaction may start at the earlier stage of lesion development, but, in general, such distinct fibre formation was missing in all the early control cases of nematodal malacia. The distinct formation of glial and mesenchymal fibres in and around cavitations, sometimes with deformity of portions of the brain or spinal cord, must be regarded as belonging to a reaction of considerably longer duration. For all these reasons, all four cases described above were concluded to be those of infection and reinfection, even though in three cases no history of any previous infection was given by the owners. The fact that the animals can be without discernible nervous residual signs, even when they show distinct pathological changes at autopsy, is in line with classical observations by physiologists. Experimental animals, such as dogs or monkeys, can be re-established functionally within three weeks, after ablation of part of the cortex, or after cutting of the lateral column of the spinal cord.

Clinically Silent or Inapparent Cases

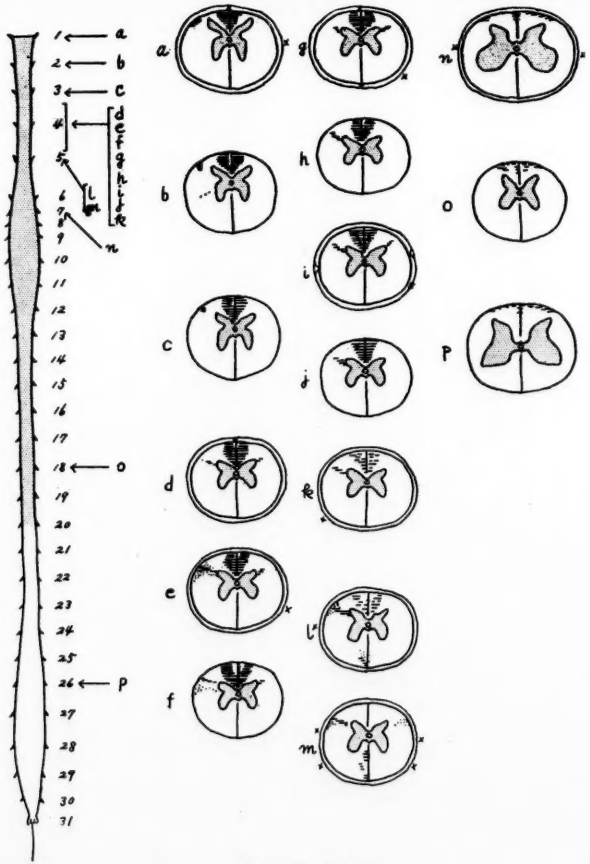
Attention was drawn previously to the fact that, in all domestic animals in which this nervous disease has been observed, and clearly identified as being caused by a nematode, the mass volume of each irregular malacic lesion in the nervous system is approximately the same. We stressed the apparent haphazard distribution of lesions in the nervous system, covering a large number of cases, regardless of whether these have been seen in animals in Japan, Korea or Ceylon. There did, however, seem to be some predilection for parasites to locate and cause

damage in the cervical portion of the spinal cord, in the thalamus, midbrain, and cerebellum. The lesion might again, however, be solely restricted to the lumbar cord, were affected by disruption of motor tracts. Even in cases with for the unfortunate clinical designation of lumbar paralysis—i.e., to those cases in which only parts of the body, below the level of a lesion in the thoracic or lumbar cord, were affected by disruption of motor tracts. Even in cases with lesions in the more cranial segments of the spinal cord, or in the brain, the animal may be paralysed completely and still lying on its side. It must be manifest that in animals such as horses with a grossly more voluminous central nervous system than that of sheep or goats; dependent on the neuro-anatomical location of a lesion, far less disruption of neurological function might be expected to occur. This indeed is in agreement with what has been experienced in the cases of horses in the Orient as the following records show. The presence of a nematode parasite with a nervous lesion is not necessarily concomitant with the onset of neurological signs. This need not be surprising, for it accords with facts about the relative immaturity of the development of the brain of the lower animal compared with that of primates. Experiences by physiologists furnish us with supporting experimental evidence, and recent advances in brain surgery partly confirm this also in human beings. The possible occurrence of " clinically silent lesions " is of importance from another angle. As Innes has stressed many times, the infrequency with which gross, naked-eye changes are found in many nervous diseases of animals has never been properly appreciated, and is brought out repeatedly—nor has the fact that a cheese-paring random selection of tissue from here and there in brain and cord might be waste of time and labour for pathological studies. In those countries where virus encephalitis does exist, it is obvious that differential clinical diagnosis alone might have little worth; without adequate examination of large areas of the brain and cord, a totally false impression might be received regarding the nature of any disease present. By the identification of cerebrospinal nematodiasis in horses, we must be aware of the possibility of the occurrence of dual infection—viz., nematodiasis and virus (or even bacterial) encephalitis—and perhaps one might set the other in motion, as recently proved by the work of Mochizuki *et al.*, on Jap B encephalitis virus and larvæ of *Toxocara canis* in experimental mice. According to research on Jap B virus encephalitis by Japanese and American workers, one might expect the occurrence of Jap B encephalitis in all three species (sheep, goats and horses) susceptible to nematodiasis; but the present authors have never found pathological changes indicative of those caused by virus in material from sheep or goats in the arthropod-borne encephalitis season in Japan. Therefore, we are of the opinion that active flagrant disease caused by Jap B encephalitis virus may not actually occur in these two animal species. Meanwhile, in horses dual infection by Jap B encephalitis virus and nematode has been reported by Sukawa *et al.* (1949).

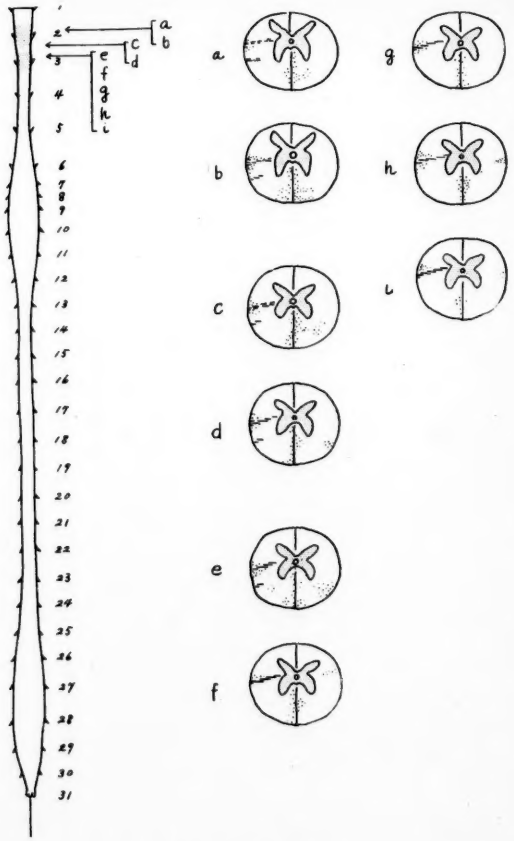
Evidence of clinically silent cases of nematodiasis in horses was well illustrated by the work of Tanaka *et al.* (1945). Examination of the CNS for lesions produced by nematodes in 100 horses used for serum production was

PLATE I

PLATE I



Case I, No. 5

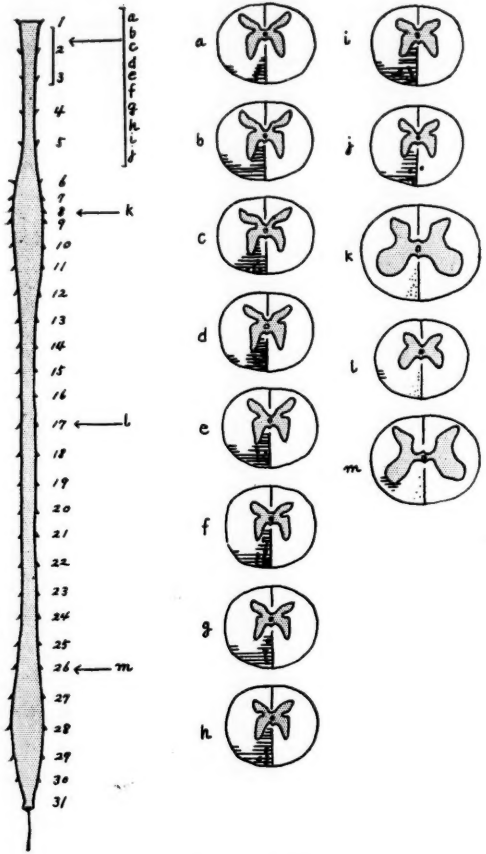


Case II, No. 11

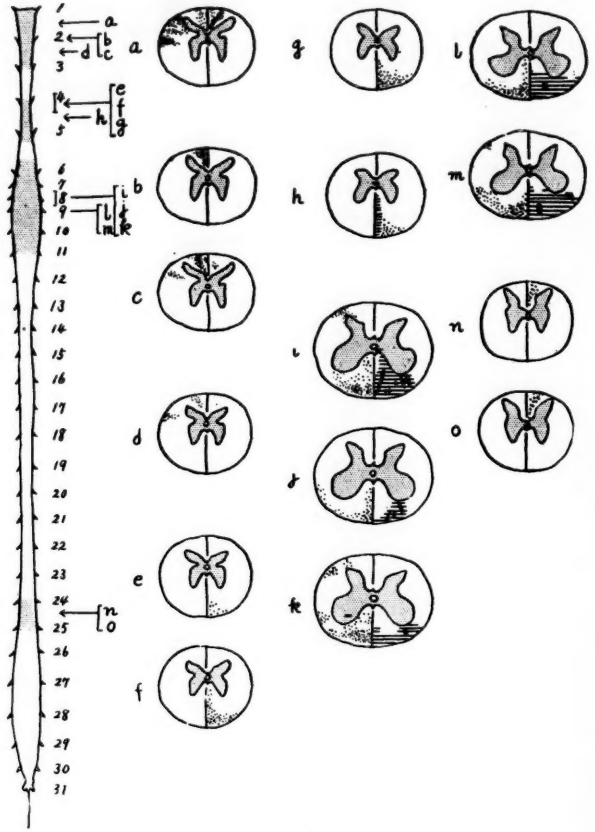
The separate diagrams attempt to depict the pathological findings in the spinal cord of the four ovine cases mentioned in this paper. Shaded portions of cord represent areas with lesions, unshaded contain no lesions; fine dots represent secondary degeneration; larger black spots and streaks represent primary malacia and microcavitation; transverse lines indicate sclerotic lesions; small crosses indicate focal eosinophilic infiltrations in, on and outside dura mater.

(*Article by Shoho and Tanaka, page* 102)

PLATE II



Case III, No. 27



Case IV, No. 31
View of the caudal cut surface.

(*Article by Shoho and Tanaka, page* 102)

induced by the previous work of Oguni *et al.* (1944), Ishii *et al.* (1944), and Fukano *et al.* (1944), on " lumbar paralysis " of foals. In an examination of the 100 brains (with 20 spinal cords) of those " serum horses," which had showed *no detectable nervous signs whatsoever* during the course of immunisation, they found lesions of nematodiasis in nine cases, and a worm was found in a cortical malacic lesion in a horse killed in November; the remainder were clearly lesions of much longer duration and chronicity. Macroscopically, these lesions were detectable as pale orange-yellowish small foci of various shapes, mostly with crevices in the white matter, or irregularly so in the cortical grey matter. In nearly all cases, microcavitations were detectable simply by aid of a magnifying glass. The predilection for location was in the cerebral cortex, the *capsula externa,* or near the *claustrum* region. In the *medulla oblongata* and deep in the cerebellar white matter some smaller lesions were detected. Microscopically, in one case, only simple tissue loss was found, and around it there was still no cellular reaction, but slight hæmorrhage was found in the microcavitation. In the one case with a parasite, the lesion occupied the *capsula externa* and its outlying cortical layer at the level of *nucleus lentiformis.* Around scattered interlacing cavities in these lesions a fine fibrillary network with few *Fettkörnchenzellen* (scavenger cells laden with fat), eosinophils, and pigment-bearing macrophages were present. The softened area, with a parasite, was full of *Fettkörnchenzellen,* and in the adjacent tissue there was some perivascular lymphocytic infiltration. Attempt at reparation was also evident in some areas around the margins by the increased connective tissue and glial fibres. The lesions in other cases were all of still longer duration, showing cavities, lined with loose network of fibres containing few cells. Gliosis seemed to be more distinct in long-standing cases. Histologically, all these cases showed lesions with communications to the outside pia, which indicated a migratory tracking-in and out by the worm. The similarity of these lesions to those found in sheep cerebrospinal nematodiasis indicated that all were caused simply by the mechanical damage due to the migration of a parasite, one of which was found in one horse. Recent experiments by Innes on needle-stab trauma of the cord of sheep proved that the nematodal lesion, found in sheep and horse cerebrospinal nematodiasis, is identical with the changes produced by mechanical trauma, and supports our views on the fundamental pathology of this disease. Clinically silent cases of cerebrospinal nematodiasis among horses might consequently be far more frequent in Japan than commonly assumed.

Comment. The existence of clinically silent cases in domestic animals with a more voluminous CNS was first made clear by simple, but meticulous, pathological examination of the whole CNS. For many workers, the presence and the identity of the worm found may be the unique criterion towards definitive diagnosis, but, in our opinion, the pathological change is enough to establish a diagnosis, for no causal agents other than *Setaria digitata* have ever been found in Japan to cause an identical disease in the horse. Evidence on the causal *Setaria,* brought out by other workers on lumbar paralysis of foals, support our views. As far as sheep are concerned, identical lesions of the same funda-

mental neurologic type were described in the U.S.A. by Kennedy, Whitlock and Roberts (1952), but by a new species of nematode—designated by them as *Neurofilaria cornelliensis*. In Japan, all worms found in cerebrospinal nematodiasis of horses, sheep or goats have been Setaria and mostly of *Setaria digitata*. The exact identity of the worms found in the disease of goats and horses in Ceylon by Innes and Pillai (and McGaughey, 1951) remains to be clarified.

Summary

Beliefs on the occurrence of repeated infections, in successive seasons, of cerebrospinal nematodiasis, which had been noted commonly by farmers in Japan, are supported by pathological evidence. The nature and varied distribution of fresh malacic foci and old-standing sclerotic lesions, both with secondary degeneration following in the train of fibre tract destruction, can yield a perplexing picture, unless the whole nervous system is examined carefully. Reinfection of sheep in the same or successive years points to the fact that there can be no development of immunity against migration of the nematodes concerned. This harmonises with a recent paper by McFadzean (1953) which indicates that immunity responses in filarial infections are low. This also serves to emphasise that prophylactic treatment, which Shoho has shown can be effective by use of the filaricide (Caricide, Lederle), may require to be repeated annually in those areas where the disease is enzootic.

Th problem of clinically silent, or masked, infection by nematodes is more clear-cut and has been demonstrated by Tanaka *et al.* (1945). This should not be underestimated, because of the potentiality of more than a coincidental relationship between neurotropic viral disease and cerebrospinal nematodiasis.

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SOME STUDIES ON THE ANTI-SULPHONAMIDE ACTIVITY OF P-AMINOBENZOIC ACID AND RELATED COMPOUNDS IN EXPERIMENTAL PASTEURELLOSIS

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STUDIES on the Anti-Sulphonamide Activity of certain chemicals are important not only in relation to the use of the sulphonamide compounds in the treatment of infections but also for the understanding of the mode of action of these chemotherapeutic agents.

In view of the special interest taken in p-aminobenzoic acid and its antagonistic effect on sulphanilamide, it was decided to study this compound with the object of determining:

(1) Whether p-aminobenzoic acid possesses any bacteriostatic effect on *Pasteurella septica.*

(2) Whether p-aminobenzoic acid could inhibit the bacteriostatic action of sulphanilamide on these organisms *in vitro.*

(3) Whether p-aminobenzoic acid could interfere with the therapeutic effect of sulphanilamide on these organisms *in vivo.*

(1) The Bacteriostatic Effect of p-Aminobenzoic Acid on Pasteurella septica

P-aminobenzoic acid was incorporated in melted ordinary agar in the following concentrations: 1/500, 1/1,000, 1/2,000, 1/4,00, 1/8,000 and 1/16,000. The agar was then poured into plates and each plate was divided into three portions, each of which was inoculated with one of the pasteurella strains tested (*Past. boviseptica, aviseptica* and *equiseptica*), using as an inoculum a loopful of a 24-hours broth culture. A control plate containing no p-aminobenzoic acid was treated in the same way. The plates were incubated at 37° C. and examined after 48 hours. The result is shown in Table I, from which it is seen that the pasteurella organisms are slightly affected by this compound, growth being inhibited at concentrations stronger than 1/2,000. In lower concentrations growth was as good as in the controls.

TABLE I

Strain	Concentration of p-aminobenzoic acid						Control
	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	
P. boviseptica	-	-	+++	+++	+++	+++	+++
P. aviseptica	-	-	+++	+++	+++	+++	+++
P. equiseptica	-	-	+++	+++	+++	+++	+++

The *pH* of the agar containing the various concentrations of p-aminobenzoic acid was checked with chlorophenol red and phenol red as indicators and the result is shown in Table II, from which it is seen that p-aminobenzoic acid in 1/500 and 1/1,000 concentrations turned the agar acid.

TABLE II

Concentration of p-aminobenzoic acid	Hydrogen ion concentration
1/500	pH 5.4
1/1000	" 6.0
1/2000	" 6.8
1/4000	" 7.1
1/8000	" 7.4
Ordinary agar (Control)	" 7.5

In order to determine whether the inhibitory effect of the compound was due to the inhibition of the p-aminobenzoic group or to the mere alteration of pH of the medium by the carboxylic radicle, the equivalent quantity of NaOH was added in order to have exact neutralisation and the sodium salt was thus obtained.

1 c.c. N/1 NaOH=0.137 gm. p-aminobenzoic acid.

A 5 per cent solution of sodium p-aminobenzoate was prepared from which various concentrations in ordinary agar were made. The bacteriostatic effect of the salt was tested as before but in concentrations ranging from 1/200 to 1/6,000 in the same way and proved to possess no inhibitory effect on the growth of *Pasteurella septica*.

It is thus seen that any bacteriostatic effect exhibited by p-aminobenzoic acid is due to its acidity and not to a direct action of the p-aminobenzoic group.

For the sake of comparison, the bacteriostatic effect of benzoic acid and sodium benzoate on pasteurella was also tested in the same way and in the same concentrations. As it is seen these compounds only differ from those already tested in that they are devoid of the amine radicle. The results showed that the bacteriostatic action of benzoic acid is more marked than that of p-aminobenzoic acid, the former causing complete inhibition at a concentration of 1/2,000 and higher although the *pH* is the same in both acids at the same dilutions. Similarly, sodium benzoate was bacteriostatic at 1/200 concentration in contrast with

sodium p-aminobenzoate which possesses no inhibitory effect at all. The bacteriostatic effect of sodium benzoate, evidently, is due to the direct action of the benzoic molecule.

It may be concluded that while the benzoic molecule possesses some bacteriostatic action, the p-aminobenzoic molecule is devoid of any.

(2) The Inhibition of the Bacteriostatic Action of Sulphanilamide on Pasteurella boviseptica and other Organisms by p-Aminobenzoic Acid

The minimum amount of p-aminobenzoic acid necessary to promote full growth in the presence of a standard concentration of sulphanilamide was not determined. *P. boviseptica* was used and the inoculum throughout the experiment was a loopful of a 24-hours broth culture previously diluted a thousand times in broth.

A 100 c.c. quantity of broth containing 1/1,000 sulphanilamide, a concentration which proved to be bacteriostatic, was divided into ten equal amounts in test tubes of 10 c.c. each. Sufficient quantities of p-aminobenzoic acid solutions were added to nine of these so as to give final concentrations ranging from 10^{-3} up to 10^{-11}. No p-aminobenzoic acid was added to the tenth tube and a second control tube consisting of broth alone was also used. All the tubes were inoculated with the organism, incubated at 37° C., and readings taken daily for three days.

Five other organisms were also tested in the same way, viz., *Streptococcus hæmolyticus, Streptococcus fæcalis, Staphylococcus aureus, Proteus X19* and *E. Coli.*

It was found (Table III) in the case of Pasteurella, that at the concentration of 10^{-3} of both compounds there was no growth, whereas in those tubes containing the same concentration of sulphanilamide and from 10^{-4} up to 10^{-9} p-aminobenzoic acid, growth was as abundant as in the control containing no drugs. In other words, p-aminobenzoic acid in these concentrations interfered with the bacteriostatic effect of 10^{-3} concentration of sulphanilamide. Thus the minimal concentration of p-aminobenzoic acid required to overcome this inhibition was one millionth of the concentration of sulphanilamide used. Beyond the concentration of 10^{-9} p-aminobenzoic acid, growth was absent, as it was in the control containing sulphanilamide alone, showing that the quantities of p-aminobenzoic acid in those tubes were too small to cause any interference with the action of sulphanilamide.

The absence of growth in the first tube containing 10^{-3} p-aminobenzoic acid is evidently due to its acidity (*pH* 6.0), which was shown before to be bacteriostatic. In the other tubes which contained lower concentrations, the reactions of the medium were gradually getting adjusted and the acidity factor thus eliminated.

In the case of other organisms tested, as is seen from Table III, the ratios of p-aminobenzoic acid required to antagonise sulphanilamide were: 1 : 1,000,000 for *E. coli,* 1 : 1,000,000 for *Proteus X19,* 1 : 100,000 for *Staphy-*

lococcus aureus, and 1 : 100 for *Streptococcus hæmolyticus*, compared with 1 : 1,000,000 for *Pasteurella septica*.

The anti-sulphanilamide activity of sodium p-aminobenzoate was also tested. The same procedure was adopted substituting sodium p-aminobenzoate for p-aminobenzoic acid. Practically the same results were obtained, except that the

TABLE III

The effect of p-aminobenzoic acid on the bacteriostatic action of sulphanilamide

Organism	Period of incubation	10^{-3} sulphanilamide in all tubes. Concentration of p-aminobenzoic acid: 10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	0	Control
Strept. faecalis	24 hrs	+	+++	+++	+++	+++	+++	+++	+++	+++	+	+++
	48 hrs	+	+++	+++	+++	+++	+++	+++	+++	+++	++	+++
	72 hrs	+	+++	+++	+++	+++	+++	+++	+++	+++	++	+++
B. coli	24 hrs	-	+++	+++	+++	+++	+++	+++	+	-	-	+++
	48 hrs	-	+++	+++	+++	+++	+++	+++	+++	-	-	+++
	72 hrs	-	+++	+++	+++	+++	+++	+++	+++	-	-	+++
Pasteurella septica	24 hrs	-	+++	+++	+++	+++	+++	+	-	-	-	+++
	48 hrs	-	+++	+++	+++	+++	+++	+++	-	-	-	+++
	72 hrs	-	+++	+++	+++	+++	+++	+++	-	-	-	+++
Proteus X 19	24 hrs	+	+++	+++	+++	+++	+++	++	-	-	-	+++
	48 hrs	+	+++	+++	+++	+++	+++	+++	-	-	-	+++
	72 hrs	+	+++	+++	+++	+++	+++	+++	-	-	-	+++
Staph. aureus	24 hrs	-	+++	+++	+++	+++	+	-	-	-	-	+++
	48 hrs	-	+++	+++	+++	+++	+++	-	-	-	-	+++
	72 hrs	-	+++	+++	+++	+++	+++	-	-	-	-	+++
Strept. haem.	24 hrs	-	+	+	-	-	-	-	-	-	-	+++
	48 hrs	-	+++	+++	-	-	-	-	-	-	-	+++
	72 hrs	-	+++	+++	-	-	-	-	-	-	-	+++

- = No growth

+++ = Heavy growth

interference with sulphanilamide action was even more marked as it extended to the following tube for each organism and that growth was abundant in the first tubes containing 10^{-3} of both compounds; thus confirming that high concentrations of p-aminobenzoic acid are bacteriostatic in virtue of their comparatively high acidity.

With the object of determining whether the benzoic molecule plays any role in sulphanilamide inhibition, it was decided to repeat the same experiments substituting benzoic acid and sodium benzoate for p-aminobenzoic acid and its salt.

It was found that these compounds had no interference with the action of sulphanilamide since bacteriostatis was evident in all tubes. Apparently this is due to the fact that benzoic acid and its salt are devoid of the amino radicle. In this place, it may be recalled that when their bacteriostatic action was tested they were found to be more powerful in this respect than p-aminobenzoic acid and its salt. Indeed sodium p-aminobenzoate has no bacteriostatic action.

Therefore, while the presence of the amino radicle renders the compound less bacteriostatic, it makes it capable of antagonising sulphonamide action.

p-Aminobenzoic acid as a possible Growth-stimulant

If interference with sulphanilamide is due to growth promotion one might be tempted to believe that p-aminobenzoic acid might cause its antagonism by promoting growth.

To determine whether such growth promotion occurs, dilutions of 1 :2,000, 1 :64,000 and 1 :256,000 of p-aminobenzoic acid in agar were prepared and poured into plates. On these plates *Pasteurella boviseptica* was seeded to observe the nature of the surface growth. A control agar plate containing no p-aminobenzoic acid was similarly inoculated for the sake of comparison.

The same technique and concentrations were also adopted but substituting shake cultures for surface inoculation with the object of making viable counts. The inoculum was 0.1 c.c. of a 24-hours broth culture diluted 100,000 times in broth. The result was recorded after 48 hours' incubation at 37° C., and is shown in Table IV, from which it is seen that p-aminobenzoic acid had no significant effect on the growth of the organism, since in all these concentrations the viable counts and the size and appearance of the colonies were almost identical with those on the control.

TABLE IV

Concentration of p-aminobenzoic acid						Control	
1:2000		1:64,000.		1:256,000			
Surface growth	Average count	Surface growth	Average count	Surface growth	Average count	Average growth	Average count
+++	60	+++	80	+++	57	+++	72

Thus, while 72 colonies were counted on the average in the control agar plates, 60 colonies, 80 colonies and 57 colonies on the average were counted in the plates containing the various concentrations of p-aminobenzoic acid. In the case of the surface growth, no difference whatsoever could be detected.

It may therefore be noted that although p-aminobenzoic acid is a most

active sulphanilamide inhibitor, it does not induce growth stimulation and hence its anti-sulphanilamide activity cannot be due to a growth promotion property. This is in accordance with the results obtained by Woods (1940) and Lockwood and Lynch (1940) who also observed that p-aminobenzoic acid does not stimulate bacterial growth.

(3) The effect of p-aminobenzoic acid on sulphanilamide therapy in experimental Pasteurella septica infections of pigeons and rabbits.

Pigeons:

Method of infection:

A virulent strain of *Pasteurella aviseptica* was grown in ordinary broth for 24 hours. Dilutions were made in broth and the M.L.D. was determined and found to correspond to 1 c.c. of 1 :500 million dilution. Sixty young pigeons weighing about 300 grams each were inoculated intraperitoneally with one M.L.D. The birds inoculated were then divided into five batches of twelve pigeons each and placed in separate cages.

Batch 1 consisted of pigeons that were treated with sulphanilamide alone after infection.

Batch 2 consisted of pigeons that were treated with sulphanilamide alone both before and after infection.

Batch 3 consisted of pigeons that were treated with a mixture of sulphanilamide and p-aminobenzoic acid after infection.

Batch 4 consisted of pigeons that were treated with a mixture of sulphanilamide and p-aminobenzoic acid both before and after infection.

Batch 5 consisted of control pigeons that were infected and left without treatment.

Method of treatment:

Granulated sulphanilamide was given to the pigeons by mouth. The prescribed dose was incorporated in some paste made of flour and moulded in the form of pills to avoid any waste.

The general scheme of treatment in batch 1 was to give the birds 150 mg. of sulphanilamide per 300 gm. of body weight immediately after infection followed by 100 mg. after five hours and thereafter 100 mg. of the drug per 300 gm. of body weight per day in divided portions morning and evening for five days.

In batch 2 the pigeons were given 150 mg. of sulphanilamide three hours before infection, 50 mg. immediately after infection, followed by another dose of 50 mg. after five hours and thereafter 100 mg. of the drug per day in divided portions morning and evening for the same period of treatment. The total dosage of sulphanilamide was thus the same as that given to the birds in batch 1.

In batch 3 the birds were given 150 mg. of sulphanilamide and 40 mg. of p-aminobenzoic acid immediately after infection, followed by 100 mg. of sulphanilamide and 20 mg. of p-aminobenzoic acid after five hours and the same amount per day in divided portions morning and evening.

The pigeons in batch 4 were given 150 mg. of sulphanilamide and 40 mg. of p-aminobenzoic acid three hours before infection, 50 mg. of sulphanilamide and 10 mg. of p-aminobenzoic acid immediately after infection, followed by a similar dose of the drugs after five hours and thereafter 100 mg. of sulphanilamide and 20 mg. of p-aminobenzoic acid per day in divided portions morning and evening during the period of treatment. As it is seen, the total dosage of the two drugs was the same as that given to the birds of batch 3.

During the five days of treatment as well as during the following five days, all the birds were put under observation and deaths recorded daily. Post-mortem examination was made on the dead pigeons which revealed the typical picture of avian pasteurellosis and the organism was recovered from them.

The result of the experiment is shown in Table V from which it is seen that while all control birds of batch 5 died in 24 hours, out of the twelve pigeons in batch 1, i.e., those infected and subsequently treated with sulphanilamide, six died in 24 hours, three died on the sixth day and three died on the eighth day after infection. All the birds of batch 2, i.e., those treated with sulphanilamide, both before and after infection, survived.

All the birds of batches 3 and 4 that were treated with a mixture of sulphanilamide and p-aminobenzoic acid died in 24 hours, behaving exactly like those of the untreated controls and demonstrating the antagonistic effect of p-aminobenzoic acid on sulphanilamide therapy. This result conforms with the results obtained by Selbie (1940) and Findlay (1940) who experimented with mice, the former with streptococcal infection and the latter with the virus of lymphogranuloma venereum.

TABLE V

Batch	Total dosage in mg. per kilog. body wt		Number of rabbits	Number of rabbits dying on day										Survived 10 days
	Sulphanilamide	p-aminobenzoic acid		1	2	3	4	5	6	7	8	9	10	
1	1700	0	12	9	3	-	-	-	-	-	-	-	-	0
2	1700	0	12	-	6	6	-	-	-	-	-	-	-	0
3	1700	400	12	12	-	-	-	-	-	-	-	-	-	0
4	1700	400	12	12	-	-	-	-	-	-	-	-	-	0
5	0	0	12	12	-	-	-	-	-	-	-	-	-	0

From this experiment it may be noted that the therapeutic effect of sulphanilamide in avian pasteurellosis is slight, just amounting to a marked delay in the death period of 50 per cent of the birds, confirming the results of Buttle, *et al.* (1937) with experimental Pasteurella infection of mice.

The prophylactic effect of the drug may be better since all the pigeons that

received sulphanilamide three hours before infection as well as after infection survived.

That p-aminobenzoic acid interfered with the effect of sulphanilamide is noteworthy.

Rabbits:

A virulent strain of *Pasteurella boviseptica* was used and the same procedure was adopted.

Sixty rabbits weighing about one kilogram each were put in similar batches. The M.L.D. was 1 c.c. of 1 :500 million dilution of a 24-hour broth culture inoculated intraperitoneally. The drugs were administereed by mouth incorporated in flour paste which the animals consumed readily.

The general scheme of treatment was much the same as that adopted in the case of pigeons with the exception of dosage which was as follows:

Rabbits of batch 1 were given 0.5 gm. of sulphanilamide per kilogram of body weight immediately after infection, followed by 200 mg. after five hours and thereafter 200 mg. of the drug per kilogram of body weight per day in divided portions morning and evening for five days.

In batch 2, the rabbits were given 0.5 gm. of sulphanilamide three hours before infection, 100 mg. immediately after infection, followed by another 100 mg. after 5 hours and thereafter 200 mg. of the drug per day in divided doses morning and evening for the same period of treatment. In this way the total dosage of sulphanilamide was the same as that given to the rabbits of batch 1.

The animals of batch 3 were given 0.5 gm. of sulphanilamide and 100 mg. of p-aminobenzoic acid immediately after infection, followed by 200 mg. of sulphanilamide and 50 gm. of p-aminobenzoic acid after 5 hours and the same amount of drugs per day in divided doses morning and evening during the five days of treatment.

The rabbits of batch 4 were given 0.5 gm. of sulphanilamide and 100 mg. of p-aminobenzoic acid three hours before infection, 100 mg. of sulphanilamide and 25 mg. of p-aminobenzoic acid immediately after infection, followed by a similar dose of the two drugs after five hours and thereafter 200 mg. of sulphanilamide and 50 mg. of p-aminobenzoic acid per day in divided doses morning and evening. The total dosage of the two drugs was thus the same as that administered to the animals in batch 3.

The rabbits were observed and a daily record of deaths was made and autopsy performed.

The result of the experiment is shown in Table VI from which it is seen that sulphanilamide has a poor curative or prophylactic effect on experimental pasteurella infection of rabbits: all died although there is a slight delay in the deaths in batch 2 in which the drug was given prophylactically and therapeutically. It was interesting to observe that p-aminobenzoic acid could

interfere with even that slight delay in the death-time effected by sulphanilamide, demonstrating again the antagonistic action of the former drug on the latter.

TABLE VI

Batch	Total dosage in mg. per 300 gm. body Wt.		Number of Birds	Number of pigeons dying on day										Survived 10 days
	Sulphan.	p-amino.		1	2	3	4	5	6	7	8	9	10	
1	750	0	12	6	-	-	-	-	3	-	3	-	-	0
2	750	0	12	-	-	-	-	-	-	-	-	-	-	12
3	750	160	12	12	-	-	-	-	-	-	-	-	-	0
4	750	160	12	12	-	-	-	-	-	-	-	-	-	0
5	0	0	12	12	-	-	-	-	-	-	-	-	-	9

Discussion

Sulphanilamide has a temporary and very slight therapeutic effect on experimental *Pasteurella septica* infections. All infected animals which were subsequently treated succumbed to the disease even though the drug was administered immediately after infection. The drug exercised a definite delay in pigeons and when given some hours before infection and continued thereafter, there was some delay in death in the case of rabbits and complete survival in pigeons. Apparently the drug has a marked prophylactic effect in avian pasteurellosis in spite of its poor therapeutic value. Buttle and his co-workers (1937) also noted the insignificant therapeutic effect of sulphanilamide in connection with experimental *Pasteurella septica* infection of mice.

When sulphanilamide was administered with p-aminobenzoic acid it caused, as would have been expected, neither prophylactic nor therapeutic effects. There was no delay in the death in rabbits nor protection in pigeons though the drugs were given before as well as after infection.

Woods (1940) suggested that the differences in sensitivity to sulphanilamide exhibited by different species of organisms are correlated with quantitative differences in ability to synthesise p-aminobenzoic acid.

The relation of p-aminobenzoic acid to the bacteriostatic effect of sulphanilamide on pasteurella is of some interest. It was found that the *minimum amount* of p-aminobenzoic acid required to counteract the bacteriostatic effect of sulphanilamide bore a ratio of 1 part of the former to 1,000,000 parts of the latter. When this ratio was compared with the ratios for other organisms tested they were found to be 1 : 10,000,000 for *E. coli,* 1 : 1,000,000 for *Proteus X 19,* 1 : 100,000 for *Staphylococcus aureus* and 1 : 100 for *Streptococcus hæmolyticus.*

When, therefore, the *ratio* of p-aminobenzoic acid to sulphanilamide for pasteurella is compared with those for other organisms, it may be noted that it is about the same as for Proteus X 19, 10 times higher than that for *E. coli,* 10 times lower than that for *Staphylococcus aureus,* and 10,000 times lower than that for *Streptococcus hæmolyticus.*

XUM

It may be feasible to explain the variations in the minimum amounts of p-aminobenzoic acid required to reverse sulphanilamide bacteriostasis for the various species of bacteria by assuming that such differences are due to differences in the amount of p-aminobenzoic acid produced by these organisms.

In the order of their capacity to produce p-aminobenzoic acid and consequently to their sensitivity to sulphanilamide, these organisms under the conditions of the experiment may be arranged as follows: *Streptococcus hæmolyticus, Staphylococcus aureus, Proteus X 19, Pasteurella septica, E. coli* and *Streptococcus fæcalis;* the last being the most active p-aminobenzoic acid producer and least sensitive to sulphanilamide.

In this work it has been shown that benzoic acid and sodium benzoate do not interfere with the bacteriostatic action of sulphanilamide. This is attributed to the absence of the amino radicle which, when present, approximates the structural formulæ of the two compounds rendering competitive inhibition for an enzyme possible. Thus it is clear that the NH_2 radicle is of great importance in the chemical structure of any compound capable of antagonising sulphanilamide.

Not only does the NH_2 radicle help in rendering a compound capable of interfering with sulphanilamide, but it also lowers to a certain extent its bacteriostatic and probably its bactericidal property. Thus, benzoic acid is definitely more bacteriostatic than p-aminobenzoic acid irrespective of any alteration in *pH* that might occur by the addition of either compound. The same was observed in connection with their sodium salts, sodium p-aminobenzoate possessing no bacteriostatic action, while sodium benzoate is quite active.

Summary and Conclusions

1. p-Aminobenzoic acid counteracts the inhibitory effect of sulphanilamide on members of the *Pasteurella septica* group of organisms as has been found in connection with other organisms by other workers. The antagonism was demonstrated both *in vitro* and *in vivo.*

2. The main characteristic feature is the extremely small amount of p-aminobenzoic acid required to abolish sulphanilamide bacteriostasis on this group of organisms as compared with the quantities required to reverse the inhibition for some other organisms. In the interpretation of this observation, is is suggested that pasteurella organisms are capable of producing p-aminobenzoic acid sufficient to account for the failure of sulphanilamide chemotherapy in experimental pasteurellosis.

3. Confirming what has been found for other organisms, p-aminobenzoic acid is not a growth-stimulant for pasteurella. Growth-stimulation and sulphanilamide inhibition do not necessarily go hand in hand as claimed by some workers.

4. The importance of the amino radicle in a compound capable of competing with sulphanilamide is discussed.

5. The loss of the amino radicle from a compound renders it bacteriostatic.

Acknowledgment

My thanks are due to Dr. M. A. Gohar, Professor of Bacteriology in the Faculty of Medicine, Cairo University, for his guidance and help in this work.

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SCROTAL HERNIA AND CRYPTORCHIDISM IN THE PIG

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SCROTAL hernia and cryptorchidism are two conditions which must cause considerable loss to the pig industry. As the two abnormalities may occur in the same litter or in successive litters (one with an inguinal ring too large and the other too small) one may suspect that there may be a connection between the two. I have failed to find any support in the literature for this contention. Both conditions occur in certain families of pigs. Certain sows continuously produce ruptured as well as rig pigs and likewise some boars seem to possess the lethal gene.

It may be that if the hereditary theory be correct abnormalities might be avoided by selective breeding methods. At the present time surgical intervention gives good results. If no steps are taken to relieve the condition the affected animals become an economic loss. A pig suffering from scrotal hernia seldom thrives. A cryptorchid pig is often the best in the litter and may only be a disappointment when the cheque is received from the bacon factory.

The Ruptured Pig

For some 30 odd years I have used the technique described by G. R. White and with excellent results. Even when pigs were of a low value, the operation was much appreciated by clients. White says that the best time to operate is when the pig is 25 to 35 lb. in weight. It has been my practice to operate soon after weaning, i.e., at 35 to 45 lb. The excellent illustration in White's book make a verbal description of the operation almost unnecessary.

XUM

It is most important to empty the alimentary tract by withholding food for 24 hours. The salient points given by White are, to suspend the animal by means of a rope looped above the hocks and suspended from a beam so that the operative field is opposite the surgeon's breast. After preparing the site, the external inguinal ring is located by pressure. An incision is then made through the skin and subcutaneous tissue down to the muscular coat of the abdominal wall. This incision should be three or four inches long and its centre should be directly over the centre of the external abdominal ring. Usually by this time the intestines have returned to the abdominal cavity. The next step is to incise the tunica vaginalis near the ring and expose the cord. By traction on the cord the testicle can usually be withdrawn from the scrotum and exposed.

The testicle is removed by severing the spermatic cord with the emasculator, and the stump of the cord is allowed to drop back into the abdominal cavity. The inguinal ring is closed by means of a purse string suture of absorbable material and the skin closed by interrupted sutures. The writer's modification of White's technique is to do a covered operation and to ligate before severing the testicle.

The aftercare recommended by White is to confine the animal in a small clean pen for ten days on a limited diet. It is not necessary to remove the sutures. While this method used to be satisfactory, under present conditions the owner does not want to be bothered with single pigs. If they are returned immediately they have recovered from the anæsthetic the litter-mates smell the blood and mutilate the operative area. On the other hand, if the pig is separated for only a few days the litter-mates appear to have forgotten it completely and vicious fighting may ensue. It is safest to isolate the patients for five or six days and then to return them in the daytime when it is possible to keep them under frequent observation.

Cryptorchid Pigs

My experience of this operation only started with the advent of stricter grading. Text books do not appear to be particularly explicit for those who are practising the operation for the first time. As with the ruptured pig a previous fast of 24 hours to empty the alimentary tract is of first importance. White recommends suspending the pig as for the scrotal hernia operation. With good anæsthesia, I have found that if an assistant sits on a chair and supports the pig by the hind legs, restraint is quite satisfactory. I use the operative site recommended by White, which is two inches below the brim of the pubis either to the right or the left of the median line. Do not make the incision too far away from the mid-line or a plexus of vessels may give trouble. White recommends an incision three inches long and penetrating all layers of the abdominal wall. The index finger and second finger are then introduced into the abdominal cavity and a search made for the testicle. In the limited number I have done I have found that with good illumination, preferably by the aid of a small electric torch, and with the intestines dropped well out of the way, it is usually possible to see the testicle or testicles or the cord and manual interference is not necessary.

In one case, a double rig, the left testicle could be seen lying at the side of the urinary bladder; when it was removed the opposite member came into view. In another case the testicles were seen lying a little further forward. In one case they were found lying just below the lowest point of the incision. In each case I have found that it is much easier to bring the testicles out with forceps than by using the fingers. The cords are usually very short and I have found it necessary to sever the non-vascular part before withdrawing the testicle through the incision. The artery forceps can then be placed on the vascular part and a ligature applied in the usual way. White recommends closing the abdominal walls with three interrupted sutures known as the through-and-through variety. He states: "All layers of the abdominal wall should be included." My method has been more conservative; I use catgut sutures for the deeper layers and nylon for the skin.

White offers two warnings:—

1. Before operating see that the testicle is not located in the inguinal canal.

This precaution is essential because if the testicle is in the canal it cannot be withdrawn through the abdominal incision.

2 Care must be exercised to avoid injury to the penis.

Anæsthesia

Since it was recommended by Slatter in 1948, I have found chloral hydrate in 5 per cent solution intraperitoneally a safe, satisfactory and economic anæsthetic for small pigs. The instructions given by the above named are as follows:

The site of injection for intraperitoneal administration is midway between the umbilicus and pubic brim. The pig is held on his back or suspended by the hind legs and the full dose is injected into the abdominal cavity. A 5 per cent solution is used and it is prepared in a 500 c.c. bottle by adding 25 gm. of chloral hydrate to enough distilled water to fill the bottle. This solution is then given at the rate of 3 c.c. per pound. (The dose is roughly 2.3 gr. of chloral per pound.)

Satisfactory anæsthesia is produced in 20 to 30 minutes and persists from one to one and one-half hours. Chloral hydrate is an irritating solution, but the slight inflammation produced soon subsides.

In rig pigs we adjust the site slightly. All pigs are weighed before injection, which is given by means of a flutter valve. Small pigs appear to be very sensitive to penetration of the peritoneum and adequate restraint and assistance is necessary in their case. An infra red lamp is of great assistance during the recovery stage.

Considerable temporary swelling usually develops after the scrotal hernia operation, but the setback after either operation appears to be negligible. A little milk is the best diet in the restricted diet stage. Most of my patients have joined their litter-mates by the tenth day and made top price for stores at auction.

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THE TOPICAL APPLICATION OF 10 per cent CHLORAMPHENICOL IN SPIRIT IN THE TREATMENT OF FOOTROT IN SHEEP

By RICHARD H. C. PENNY, B.V.Sc., M.R.C.V.S.

Leighton Buzzard

THE problem of contagious footrot has, in recent years, become one of considerable importance to the sheep industry. In certain districts, particularly those with a heavy rainfall or those with very low-lying land, it is fast becoming a major scourge, and under the adverse weather conditions of 1954 it has been particularly prevalent throughout the country. The normal treatments by the foot bath method, topical applications of formalin or copper sulphate, and the newer proprietary foot dressings, have failed to control the condition.

Stewart (1954) in Southern Australia records his results from the controlled use of a number of preparations and reports very favourably on treatment with 10 per cent Chloramphenicol (Chloromycetin) in propylene glycol. A report from this country, Sambrook (1955), confirms his results. The present writer was enabled to obtain a supply of 10 per cent Chloramphenicol in spirit for a small clinical trial and the results so far obtained are very encouraging. Treatment is simple and should create no difficulty for the average shepherd.

The largest batch of sheep treated was a bunch of fifty Kerry Hill ewes brought into this district for wintering. When purchased, the majority were found to be severely affected with contagious footrot, and a large proportion of cases involved two or more feet. There was severe softening, under-running of the horn of the soles and heels and abscess formation in the interdigital space, and, in one or two cases, of the skin horn junction. There was a typical odour in most cases. The procedure adopted followed very closely that recommended by Stewart. All feet were inspected, and the affected ones were pared carefully and the dressing applied sparingly by means of a small paint brush. The sheep were then turned into a clean concrete yard and allowed to remain there for a period of two to three hours. Dressings were carried out when the weather was fine, for the convenience of the operators and to enable the spirit to dry thoroughly. Only five cases failed to respond to one application and these were repainted fourteen days later. The product was obtained in 4 oz. bottles and it was found that one of these contained sufficient to treat approximately fifty feet.

Besides the above group of lambs, numerous small bunches or individual cases of severe footrot in both sheep and goats have been similarly treated and there have been no failures, although in many cases two applications have been required. A number of tups urgently required for service were speedily cured and were thus able to carry out their work. In all cases farmers and shepherds have been more than satisfied with results, although economic factors may damp their enthusiasm when the drug is used commercially.

Discussion

The writer records his clinical experience with 10 per cent Chloramphenicol in spirit in the treatment of contagious footrot of sheep and makes the following observations:—

1. The findings of Stewart have been confirmed.

2. The treatment is an improvement on previous methods because of the ease of application and the high percentage of recoveries. In all cases, one dressing, or at the the most two dressings, with an interval of fourteen days, brought about a speedy resolution of the condition. Relapses were rare.

3. It would seem to be essential to maintain the sheep on a dry concrete yard or wooden floor for some hours after treatment to obtain the maximum effect.

4. Economy is essential, otherwise economic factors may prevent the widespread use of this product. Four ounces was sufficient to treat approximately fifty feet.

Acknowledgment

My thanks are due to Parke, Davis & Co., Ltd., for supplies of Chloromycetin 10 per cent in spirit.

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ABSTRACTS OF CURRENT LITERATURE

FURUNCULOSIS IN NORTHERN RUSSIA: S. S. Lapidus: Veterinaria, Dec., 1954. Moscow.

IN the Komi Republic in the region of Archangel furunculosis broke out in the winter among a number of working horses; it was typified by suppuration and necrotic foci appearing on the withers, tail and along the back. Sixty per cent of the horses were soon affected, timber haulage was interfered with and the condition did not improve under ordinary treatment. Believing some relief might be obtained by the administration of sulphur, the affected animals were given this substance, made into a bolus with meal, in daily doses of 1 gm., and considerable improvement was observed a week later. Profiting from this experience, the other horses on the ranch were also given sulphur mixed with their morning oats and in addition received 20 gm. of salt with their evening meal. The affected foci cicatrising over satisfactorily, the animals were gradually returned to work as soon as it was apparent that the harness would not cause further damage. The outbreak was stamped out before the end of the winter. (It is of interest here to note that Hutyra and Marak record furunculosis as occurring in horses only in hot weather.)

R.H.M.

XUM

THE TREATMENT OF AN EXTENSIVE AND PENETRATING WOUND OF THE THORAX IN A YOUNG FOAL: Turk Veteriner Ankara. Sept.-Oct. 1954.

A SIX-DAYS-OLD filly was brought into the clinic with the right side of the thorax showing an extensive wound caused by the horns of a water buffalo. The wound was about 20 cm. long, extended above the vena thoracica between ribs 7-15 and penetrated the skin and underlying muscles; in the depths was a hole through which air could be heard passing. On investigation the parietal pleura was found to be ruptured between the seventh and eighth ribs and the lung was herniated through the rent in it, which was about 9 cm. long. The external thoracic vein was thrombosed, there was pneumothorax and pleurisy in the right side, the animal experienced difficulty in breathing with the respirations at 42 to the minute; the pulse was 72 and the general condition serious. Therapeutic measures were immediately put in hand: the prolapsed portion of the lung and adjacent part of the chest were washed with boiled water disinfected with rivanol while 1,000,000 units of crystallised penicillin were directed into the wound, the intercostal muscles were sutured with No. 4 catgut as were the muscles overlying; the skin was sutured with No. 3 silk and a pad impregnated with gasiodoform was bandaged round the chest, 20 c.c. of antitetanic serum were given together with 4,000,000 units of penicillin; 3 gr. oily streptomycin and 50 c.c. 33 per cent suphamegathine solution were also administered. This treatment was continued for five days, after which time the temperature had dropped from 40.1° C. to 38.4° C. and food and water were being taken. By another five days the wounds of the skin had healed and the sutures were removed and the animal was discharged cured 12 days after it had been admitted.

R.H.M.

NOTE SUR LES CARACTERES ANATOMIQUES DE LA COUPE MEDIANE DU STERNUM DES BOVINS ET LEUR EXPLOITATION DANS LA DIAGNOSE DE L'AGE DES ANIMAUX APRES ABATTAGE. By le Vétérinaire Commandant Le Seac'h. *Revue du Corps Vétérinaire De L'Armée.* February 18, 1954.

THE French meat inspection regulations require that beef should be from cattle aged not less than two years and not more than eight years. Difficulty is sometimes experienced in deciding the age of a carcase, more particularly when the head is no longer present and the author suggests a method of ageing the carcase by reference to the inter-sternal joints.

He notes that these joints are clearly seen in midline sections of the sternum and that, while the diathrodial joint between the first and second sternebræ persists through life, the cartilaginous plates between the remaining sternebræ are gradually replaced by bone during the fifth to tenth years. At eight years not less than two of three plates remain and he proposes this as a criterion of carcase acceptability. This view is based on observations on more than 5,000 carcases from different breeds.

K.M.D.

HISTORICAL NOTE

THE CUNEIFORM IDEOGRAPH FOR "HORSE"

By R. H. MERLEN, M.R.C.V.S.

London

As is thoroughly well known the Assyrians inherited the cuneiform writing from the Sumerians, a people who had inhabited Assyria at a very early period and who were apparently non-Semitic in origin. Originally this system of writing consisted of a series of ideograms and little stylised pictures and when it was taken over by the Semites about 1500 B.C. it was modified and adapted by them who, of course, gave Semitic phonetic values to the Sumerian stroke groups and pictures—as someone might write "cheval" but pronounce it "horse." In common with some other systems of writing, the cuneiform made use of determinatives or classifiers which were groups of strokes placed before a word and which gave an indication of the class of object or of creature to which the ensuing word belonged. As for example "ilu" (from which "el" and "Allah") always appeared before the names of the gods, and "nun" ("fish") before the names of fishes. The determinatives were not apparently pronounced in conversation. "Imeru," from which the Arabic Hamir and the Hebrew Hamor are derived, and served as a determinative for the names of three quadrupeds—gammalu the camel, paru the mule and sisu the horse—not because the Assyrians suffered from any illusion that these three species came from the same zoological family but no doubt because being all working animals they were considered best grouped together under the sign of the donkey, the apotheosis of all beasts of burden. The ideogram to which the Semitic value of "sisu" was given (and as sus the word is frequent in the Hebrew scriptures to describe a draught horse as distinct from "Feres" the charger) is composed of two stroke groups of which the second is constant in having the phonetic value "Ra" while the first may have a phonetic value "Kur," "mat," "shad," "shat," etc., or may itself be a determinative signifying "country" where it has the value "matu." While "matu" was often succeeded by "Ki" after the names of countries in and around Assyria, foreign lands such as "Mu-su-ri" (Egypt), "Kuusi" (Ethiopia) were merely introduced by the simple "matu." It follows from this that (Imeru) "sisu" may be read "(Imeru) Matu Ra," i.e., the ass from the land of Ra. "Ra potamos" was the name by which the ancients knew the Volga and Ammianus Marcellinus (Books 12 and 22) tells us that the Rha derived its name from the rha or rhubarb growing on its banks; "rha barbara" in fact gives us the word Rhubarb. This "Rha" was apparently a local name and may possibly be an ancient "Turkish" word since "Rawand," the modern Turkish for rhubarb (from which the Russian Rievien), is obviously closely connected with it. The "Entziklopedicheski Slovar" (Moscow, 1951) describes rhubarb as occurring in Transcausia and the region of the Tyanshan mountains in addition to places much further to the East; and this reference together with the allusion to the Volga in Ammianus Marcellinus would seem to indicate that the Land of Ra of the inscriptions lay between the Caspian and the modern Chinese province of

Sinkiang. It is hardly necessary to add that these regions were known somewhat vaguely in ancient times as Scythia intra and extra Imaus and were recognised again vaguely as the home of the great horse-owning tribes of nomads, the Abii, Hippemolgi—mare milkers—and others. It has always been surmised that the horse came into Assyria and elsewhere from somewhere in central Asia, but it is perhaps interesting to see this surmise apparently confirmed by this connection between the word Sisu and Matu Ra.

REVIEWS

MICROBIOLOGY OF MEATS, by Lloyd B. Jensen. Third edition. 1954. Illinois: The Garrard Press. Pp. 422 + xvi. Price 48s.

This is the third edition within the space of twelve years and the author has shown his appreciation of the increase in our knowledge of the microbiology of meat and meat foods. Meat processing was practised before laboratory aid was considered; nevertheless much new technological material on meat and meat foods has become available within recent years. The author is well qualified for his task as he is the chief bacteriologist at the Research Laboratories of Swift & Co., Chicago; he has also had experience of teaching, not only in the Universities of Minnesota and Chicago, but also at the Army Medical Service School at the Chicago Quartermaster Depot. In the preface to this edition he pays a tribute to the great benefits to the science of meat sanitation and technology which are the result of long and wide experience of the veterinarians and other scientists of the Federal Meat Inspection Service and the Bureau of Animal Industry.

There are in all fifteen chapters and two deal with the microbiology of beef and of bacon; others consider the bacteriology of the normal tissue of pigs, and of those ingredients, such as spices, sugars, paper and wood, which are concerned in processing. The production of gaseous fermentation and the action of micro-organisms on fats take up two of the chapters. The quality of meat and meat products may be determined by æsthetic considerations; the problems of abnormal colour and appearance as well as the safety of the foods for human consumption are all dealt with.

The problems of food are of a different character according to the habits and methods of behaviour of men. Clean food of wholesome appearance and available in all places at all times and at all seasons necessitates its handling, preservation and transport. To this must be added the necessity of providing products which are safe for human consumption. The distance from the living food animal to the table of the consumer may be long and tortuous. Micro-organisms constitute the greatest hazard and yet many may contribute beneficially. Their significance and the part they play are dealt with here, although it is not forgotten that preservation of food was practised before the part played by micro-organisms was appreciated. This book is a useful guide for those whose interests lie within this broad field.

FARMER AND STOCK-BREEDER YEAR BOOK, 1955. 79th Edition. London: Farmer and Stock-Breeder Publications, Ltd. 10s. 6d. net (postage 6d).

THIS old-established reference book does not need any eulogy to assert its value. It is sufficient to say that all agriculturists will find within its pages a wealth of classified statistical information which will supply the answer to almost any query.

The format changes little from year to year. The book is superbly illustrated, especially that section dealing with the pedigree year. Undoubtedly Britain has the best stock in the world.

The special articles always deal with contemporary farming problems and this year's selection concerns some of the topical subjects which are exciting the farmers' interest in these days when farming is becoming more and more dependent on its own efficiency for economic survival. List of special articles:

"Planning for Free Markets," by H. G. Sanders.
"Keeping Milk Profitable," by M. M. Cooper.
"Hill Grass Improvement," by A. J. Davies.
"Virus Pneumonia in Pigs," by Bruce Jones.
"Market Gardening on the Farm," by F. H. Garner.
"Economic Farm Fencing," by F. G. Beadon.
"Future Market for Pork," by E. Gunner.
"Care of In-pig Sow," by W. E. Coey.
"Well Presented Fleeces," by B. J. Robinson.
"A Year Among Equipment," by George Wardrop.
Poultry:
"Husbandry and Disease," by J. E. Wilson.
"Table Poultry and Eggs," by H. L. Tabor.

They deal authoritatively with many aspects of farming for the authors are recognised experts in their own particular fields. We presume that the author of the excellent article on "Virus Pneumonia in Pigs" is a veterinary surgeon. If this is so, why should the reader be denied this information?

It is a sad sign of the times that the horse is no longer considered worthy of a special article. There are many who think that he still has his part to play in the economy of the farm and we hope that our friend will not be neglected in future issues. In these days when horse mastership is at a discount and information is not gleaned so readily by precept and contact, interested youth consumes the written word with great avidity.

ITEMS OF VETERINARY INTEREST

THE Animal Health Trust announces that the first Baxter Memorial Fellowship has been awarded to Mr. A. L. Ogden, M.R.C.V.S., D.V.S.M., to enable him to carry out a survey of the problem of bovine infertility. This work will be done in close co-operation with the Milk Marketing Board and various cattle breeders.

XUM

ADVANCES in the scientific world which have been noteworthy in the past decade are having an increasing effect on the daily life of every individual. It is of particular interest to veterinary workers when such developments take practical shape in providing better equipment for field and laboratory work, but it is often the case that scientific workers themselves are the last to benefit from new discoveries as the popular demand is more insistent and meeting it more rewarding.

We were interested to see that the makers of the well-known "Pyrex" glass have produced a new double-tough "Opalware." This is two to three times as strong as any similar product and has the advantage of a stain and scratch proof surface which has been shown under ultra-violet ray tests to be bacteria-free after normal cleansing. It may be that "Opalware" in due course will add to James A. Jobling's already wide range of laboratory glassware though, at present, manufacture is being limited to catering equipment. Many veterinary surgeons will no doubt have seen a new syringe made entirely from transluscent nylon which is now being marketed by Chas. F. Thackeray, of Leeds. This is guaranteed unbreakable under normal circumstances, can be sterilised by boiling at up to 120 C., is leakproof and has outstanding compression. A new steriliser called the "Surqual Minor," which is electrically heated (or can be used with a spirit lamp) is now available from Surgical Equipment Supplies Ltd., London, W. This has an automatic cut-out to prevent boiling dry.

WE read of an outdoor poster campaign in the United States which is about to be conducted by a large veterinary manufacturing firm there. Posters in three colours are to appear throughout the country urging animal owners to "Consult Your Veterinarian Regularly." General approval seems to have been obtained from the profession for this method of educating the general public and improving relations with livestock owners. While withholding comment at this stage we certainly know of less worthy causes for poster campaigns.

SIR WINSTON CHURCHILL has received the diploma of Honorary Associateship of the Royal College of Veterinary Surgeons. This was presented to him by the President, Dr. W. R. Wooldridge, at a recent ceremony at No. 10 Downing Street.

ECHO FROM THE PAST

(*From the* VETERINARY JOURNAL, *March,* 1880)

NOTES AND NEWS.

Antiquity of the Horse on the American Continent.

SINCE the original account of American fossil horses given by Leidy, the Eocene strata of New Mexico and Wyoming have yielded two very important ungulates, which have helped to complete the history of the descent of the horse,

so well worked out by Professor Marsh. These relics carry back the ancestry of this familiar quadruped to the oldest Tertiary time. The earliest form, *Eohippus,* was about the size of a fox, had forty-four teeth, the molars having short crowns and being quite different in form from the pre-molars. There were four well-developed toes, a rudiment of another on the fore foot, and three toes behind. The structure of the feet and of the teeth in *Eohippus* indicates, beyond question, that the direct ancestral line to the modern horse had already separated from the Perissodactyls. The second of these ungulates, *Orohippus,* is from Wyoming Eocene, and is evidently next to *Eohippus,* which it now replaces in the line of descent. In size it about equalled its predecessor, but the rudimentary digit of the fore foot has disappeared, and the last pre-molar has gone over to the molar series. Another Eocene equine, discovered in Utah, is *Epiohippus.*

NOTICES

Evans Medical Supplies Ltd. have been appointed sole veterinary distributors for Comprox, a liquid detergent marketed by Irano Products Ltd., a subsidiary of British Petroleum Ltd. (formerly the Anglo-Iranian Oil Company).

Comprox belongs to the sodium alkyl sulphate group of liquid detergents, and is in a concentrated form, making it most economical to use. It is neutral in reaction, practically odourless and has a wide range of uses in dispensaries, surgeries, kitchens, kennels, inside, outside, and in fact everywhere where strict cleanliness is demanded. A descriptive leaflet has been mailed to veterinary surgeons and further are available on request from the company's establishments at Liverpool, London, Newcastle and Swansea, as well as from Evans Medical's Glasgow agents, The New Apothecaries Co. Ltd.

Imperial Chemical (Pharmaceuticals) Ltd., announce the introduction of " Hibitane " Pessaries for the prevention and treatment of metritis and endometritis in large animals. Full particulars are available to veterinary practitioners from the manufacturers at Wilmslow, Manchester.

Glaxo Laboratories announce that their stabilised injection of Streptomycin Sulphate will in future be described by the trade name " Strepolin." The product remains unchanged.

Publishers' Notices

The British Veterinary Journal, with which is incorporated The Veterinary Journal, is published monthly, and copy for advertisements should be in the hands of the advertisement manager not later than the 20th of the preceding month, if proof is required. Tel.: Temple Bar 3386.

Letters for the Journal, literary contributions, reports, notices, books for review, exchanges, new instruments or materials, and all matter for publication (except advertisements) should be addressed to the Editor.

Annual Subscription, 40s. ($6.50 U.S.A. currency, post free).

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